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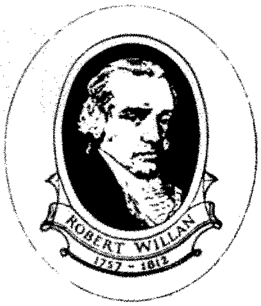

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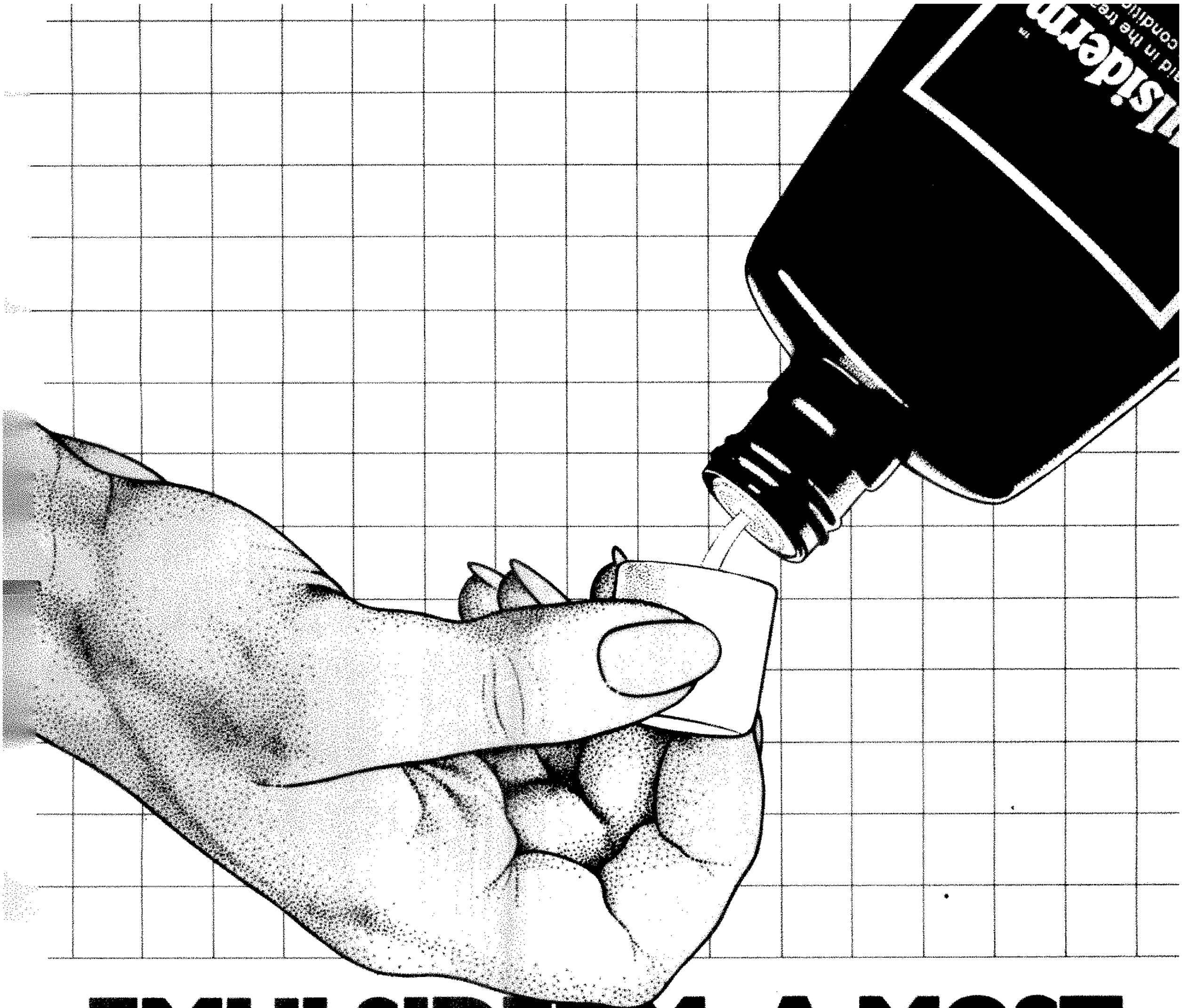

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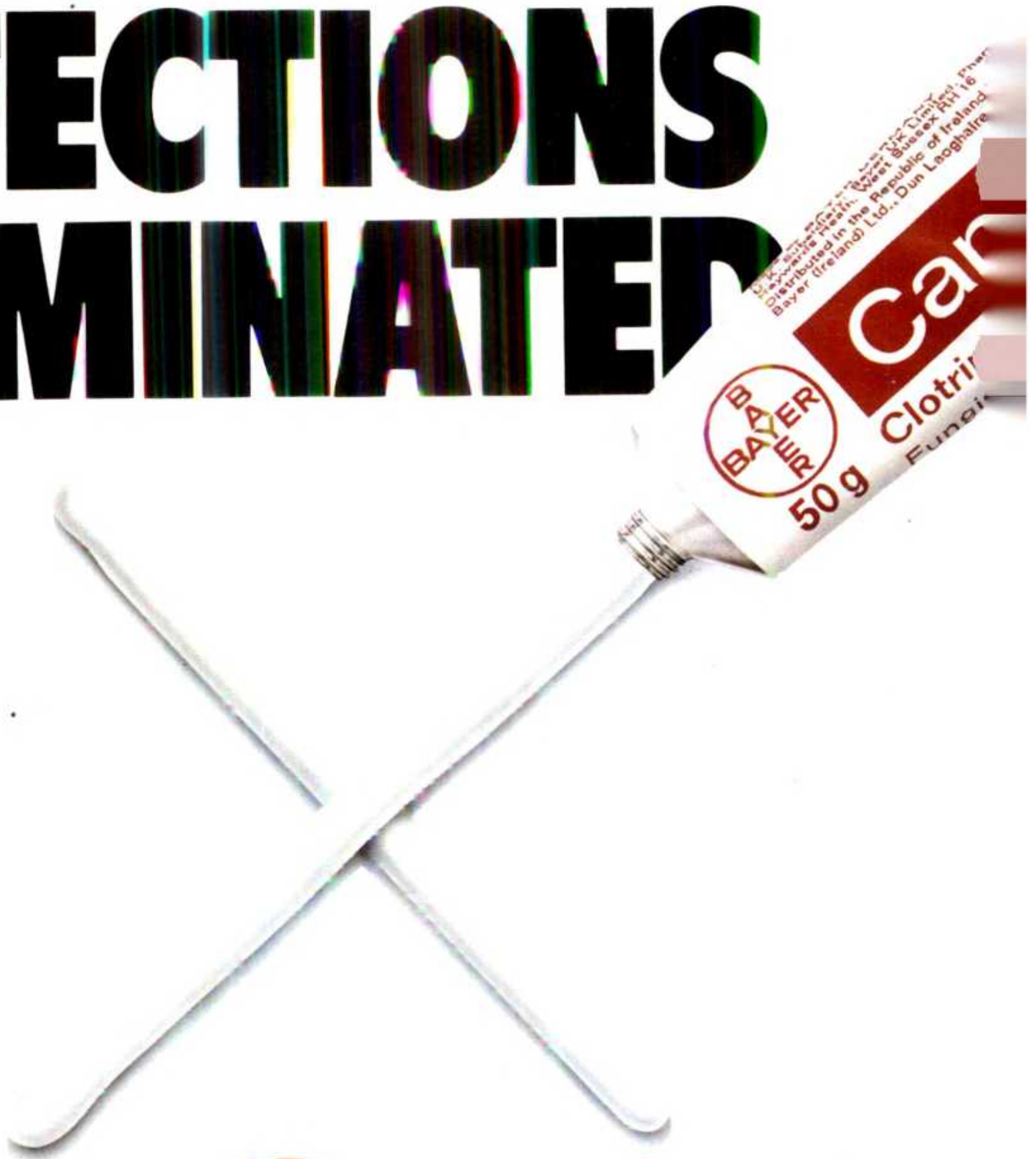
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Canesten®

clotrimazole

Established by experience

Prescribing Information:
Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Solution* A clear solution containing 1.0% clotrimazole in polyethylene glycol. *Powder* A white powder containing 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections. **Dosage and Administration** *Cream, Atomiser Spray, Solution* Apply to the affected area 2-3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic against re-infection. **Side-effects** Rarely patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near naked flame,
allowed to come into contact with the eyes, ears or mu
membranes and should not be inhaled. **Basic NHS c**
20g £1.84, *50g* £4.30. *Atomiser Spray 40ml* £5.17. *Solu*
£2.40. *Powder 30g* £1.58. **Product Licence Numbers**
0010/0016, PA21/4/2. *Atomiser Spray* PL 0010/0060,
Solution PL 0010/0082, PA21/4/3. *Powder* PL 0010/C
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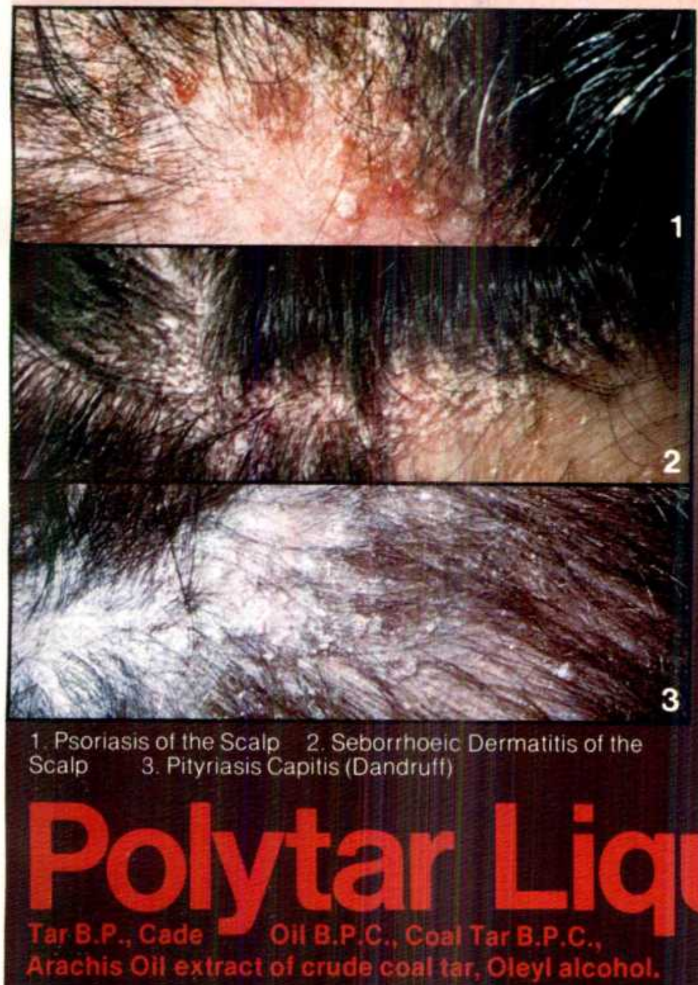
Common Disorders of the Scalp.

They all have one thing in common — the treatment



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

Polytar Liquid

Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

ALSO Polytar Plus with protein

Prescribing Information.

Presentation Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coat Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	91p
	150ml	£1.34
	350ml	£2.37
	1 litre	£6.18
Polytar Plus	150ml	£1.43
	350ml	£2.80

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

Stiefel Laboratories (UK) Limited
Wellcroft Road, Slough SL1 4AQ

Unblemished...
early treatment of acne with
● LOW-DOSE ●
Minocin
minocycline hydrochloride
50mg b.d.
helps to minimise the risk of permanent scarring

Dosage Adults and children over 12 years. One tablet twice daily. Minocin may be taken with meals. Treatment of acne should be continued for a minimum of six weeks. **Contra-indications** Known tetracycline hypersensitivity. Systemic lupus erythematosus. **Precautions** Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. **Warnings and side effects** Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discoloration of teeth is a possible sequela of treatment during pregnancy or early childhood (up to 8 years of age). **Presentation** Beige tablets containing 50mg of minocycline as the hydrochloride, supplied in bottles of 100 at a basic NHS cost of £22.44. PL 0095/0062. MINOCIN is a trademark. Full prescribing information is available on request.

Lederle **Lederle Laboratories** Division of Cyanamid of Great Britain Ltd. Fareham Road, Gosport, Hants PO13 0AS (Tel: 0329-236131)

With acknowledgements to H.M. Bateman.

THE DOCTOR WHO THOUGHT RD STOOD FOR ROYAL DOULTON.

We thought everyone knew that RD stands for Ready Diluted! And that Betnovate RD is simply a ready-prepared 1-in-4 dilution of Betnovate.

But perhaps not everyone realises all the advantages this provides: reliable and consistent quality of manufacture, no contamination or loss of efficacy in preparation and a 2-year (rather than a 14-day) shelf life.

Betnovate RD is available as cream or ointment in familiar and convenient 100 gram tubes.

Betnovate RD

(betamethasone valerate)

Simply Betnovate, Ready Diluted

Prescribing information

Presentation

Betnovate RD Cream and Ointment contain betamethasone valerate ready-prepared in a one-in-four dilution, giving a strength of 0·025%.

Uses

Inflammatory dermatoses responsive to topical corticosteroid therapy. Betnovate RD preparations are indicated for maintenance treatment when control has been achieved with Betnovate (0·1%) Cream or Ointment.

Dosage and administration

A small quantity of Betnovate RD should be applied to the affected area two or three times daily. Betnovate RD Cream is especially appropriate for moist or weeping surfaces and Betnovate RD Ointment for dry, lichenified or scaly lesions, but this is not invariably so.

Precautions

Long-term continuous topical therapy should be avoided where possible, particularly in infants and children, as adrenal suppression can occur even without occlusion. Appropriate antimicrobial therapy should be used whenever treating inflammatory lesions which have become infected. Any spread of infection requires withdrawal of topical corticosteroid therapy and systemic administration of antimicrobial agents. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Prolonged and intensive treatment with highly active corticosteroid preparations may cause local atrophic changes in the skin such as striae, thinning and dilatation of the superficial blood vessels. As with other topical corticosteroids, prolonged use of large amounts, or treatment of extensive areas, can result in sufficient systemic absorption to produce the features of hypercorticism. This effect is more likely to occur in infants and children. Betnovate RD preparations are usually well tolerated, but if signs of hypersensitivity appear, application should stop immediately.

Contra-indications

Rosacea, acne and peri-oral dermatitis. Skin lesions caused by infection with viruses (e.g., herpes simplex, chickenpox), fungi (e.g., candidiasis; tinea) or bacteria (e.g., impetigo). Hypersensitivity to the preparation.

Product licence numbers

Betnovate RD Cream: 0004/0277
Betnovate RD Ointment: 0004/0278

Basic NHS cost (exclusive of VAT)
Betnovate RD Cream/Ointment
100 gram tubes £2·90

Glaxo

Leaders in topical steroid therapy

Further information on Betnovate and Betnovate RD is available on request from Glaxo Laboratories Limited
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Betnovate is a Glaxo trade mark

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The Universal Radiation System for the treatment of

Psoriasis, Acne and other Skin Disorders

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using 3 different spectra but only one machine

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The Form of Therapy can be altered within seconds

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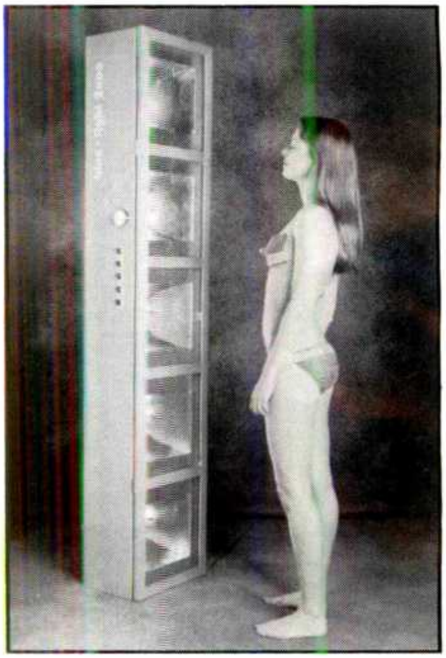

Textbook of Dermatology

Edited by **Arthur Rook** MD, FRCP *Consultant Dermatologist, Addenbrooke's Hospital, Cambridge*

D.S. Wilkinson MD, FRCP *Consultant Dermatologist, Aylesbury and High Wycombe Hospital Group*

and **F.J.G. Ebling** DSc, PhD *Professor of Zoology, University of Sheffield*

'This latest edition continues its deserved place as a comprehensive encyclopedia of the scope of dermatology with a vast array of disease states presented to the reader. All dermatologists ... and all medical libraries, should reserve a place on their bookshelves for this classic text.' *Journal of the American Academy of Dermatology*

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Third Edition, 1979. 2448 pages, 1131 illustrations.
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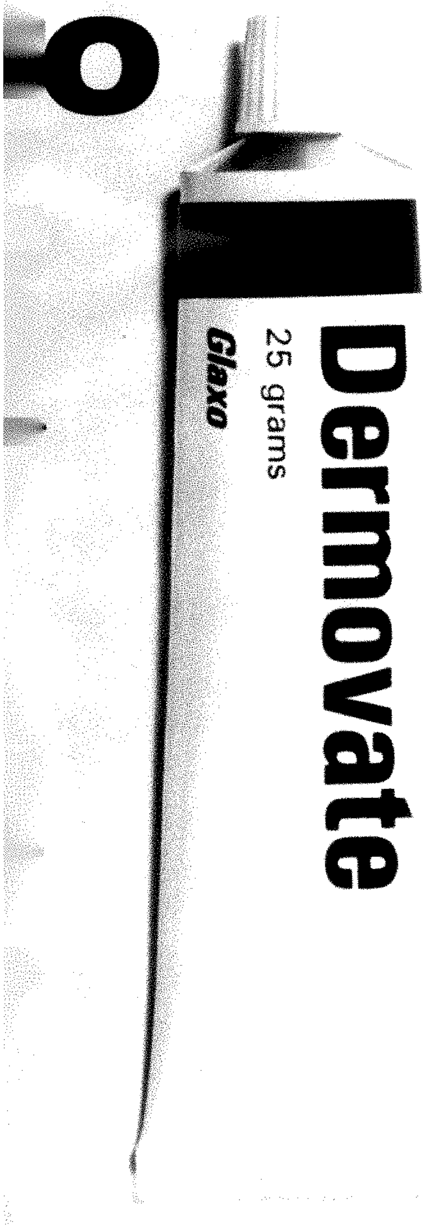
When you need
o be strong.



> ‘In chronic eczema, for example in the atopi variety, it may well be better to use strong topical steroids for short courses, e.g. two weeks, to try to clear the eczema and then sto treatment, rather than to use weaker steroids on an indefinite basis.’

Fry L. Update: Mar 1978 681-

Because it's strong . . .
it works on even long-standing, stubbor difficult dermatoses.

Because it's strong . . .
it works quickly, so minimising the risk o steroid side effects.

Because it's strong . . .
it needs to be used only sparingly and infrequently.

Because it's strong . . .
it often gives prolonged periods of remission.

Dermovate
(clobetasol propionate)

Its strength is its strength

bing information

and administration
ringly once or twice daily until improvement occurs. t should not be continued for more than four weeks view.

ects
pical corticosteroids local atrophic changes may ccur following prolonged and intensive treatment longed use of large amounts or treatment of areas may produce the features of hypercorticism. re likely to occur in infants and children, and with . In infants, the napkin may act as an occlusive

the weekly dosage of Dermovate is less than 50 dults, any pituitary-adrenal suppression is likely to be ith a rapid return to normal values once the short teroid therapy has ceased. The same applies to ven proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.
Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications
Rosacea, acne and peri-oral dermatitis. Bacterial, fungal or viral diseases of the skin. Hypersensitivity to the preparation.

Basic NHS cost
(exclusive of VAT)
Dermovate Cream or Ointment 25 gram tubes £2.07 (als available in 100 gram tubes).

Presentation	Product licence number
Dermovate Cream:	4/0219
Dermovate Ointment:	4/0220

Glaxo Leaders in topical steroid therapy

Further information on Dermovate is available from:
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ENSURES PATIENT ACCEPTANCE

Now...clear elegance in a therapeutic tar shampoo!

T/Gel
Shampoo
Relieves itching, flaking scalp with regular use.
Aids in the treatment of scalp psoriasis, dandruff and seborrheic dermatitis.
125 ml
Neutrogena®
T/Gel
Shampoo
Relieves itching, flaking scalp with regular use.
Aids in the treatment of scalp psoriasis, dandruff and seborrheic dermatitis.
125 ml
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T/GEL is a new medicated shampoo formulated as effective everyday therapy for scalp psoriasis, dandruff and seborrheic dermatitis. Regular use helps control itching, flaking and scaling while leaving the hair lustrous and manageable. T/GEL contains Neutar, a coal tar extract, equivalent in potency to 2% crude tar in suppressing D.N.A. synthesis (cell turnover). The unique clear T/GEL formula gives a rich lather, is pleasant to use, and free from lingering tar odour, encourages patient compliance.

PRESCRIBING INFORMATION

PRESENTATION T/GEL is a concentrated medicated tar based shampoo containing Neutar a solubilised coal tar extract 2% in a bland shampoo base consisting of: purified water, USP; sodium laureth sulfate; cocamide DEA; cocamidopropyl betaine; fragrance; imadazolidinyl urea; methylparaben; propylparaben; tetrasodium EDTA and citric acid.

USES Indicated as an effective aid in the treatment of psoriasis, dandruff and seborrheic dermatitis.

DOSAGE AND ADMINISTRATION Hair should be wetted thoroughly and a liberal amount of T/GEL applied and massaged into scalp, allow lather to remain on scalp for several minutes, then rinse, repeat application and rinse thoroughly.

PACK AND PRICE Pack 125 ml. Basic NHS price £1.52

PRODUCT & LICENCE NUMBER 5165/0001

Neutrogena®

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Neutrogena (U.K.) Ltd., P.O. Box 27,
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The simple solution to troublesome scalp conditions

so easy to use

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betamethasone valerate

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Betnovate Scalp Application is presented in a plastic squeeze bottle with an elongated nozzle. The pack is specially designed so that the alcohol/water based steroid can be conveniently and easily applied direct to scalp lesions.

The preparation has an immediate cooling effect and gives rapid relief from the itching and inflammation associated with seborrhoeic dermatitis and other troublesome scalp conditions.

Betnovate Scalp Application is colourless, odourless and does not stain or leave unsightly residue in the hair.

Specially formulated; specially packed to make treatment easy

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until improvement
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areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism. This is more likely to occur in infants and children with occlusion, or when treatment is prolonged. Local atrophy may occur after prolonged treatment under occlusion. Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Basic NHS cost (exclusive of VAT)
30ml – £1·70
Product Licence number
4/5133

Further information on Betnovate (trade mark) Scalp Application is available from:
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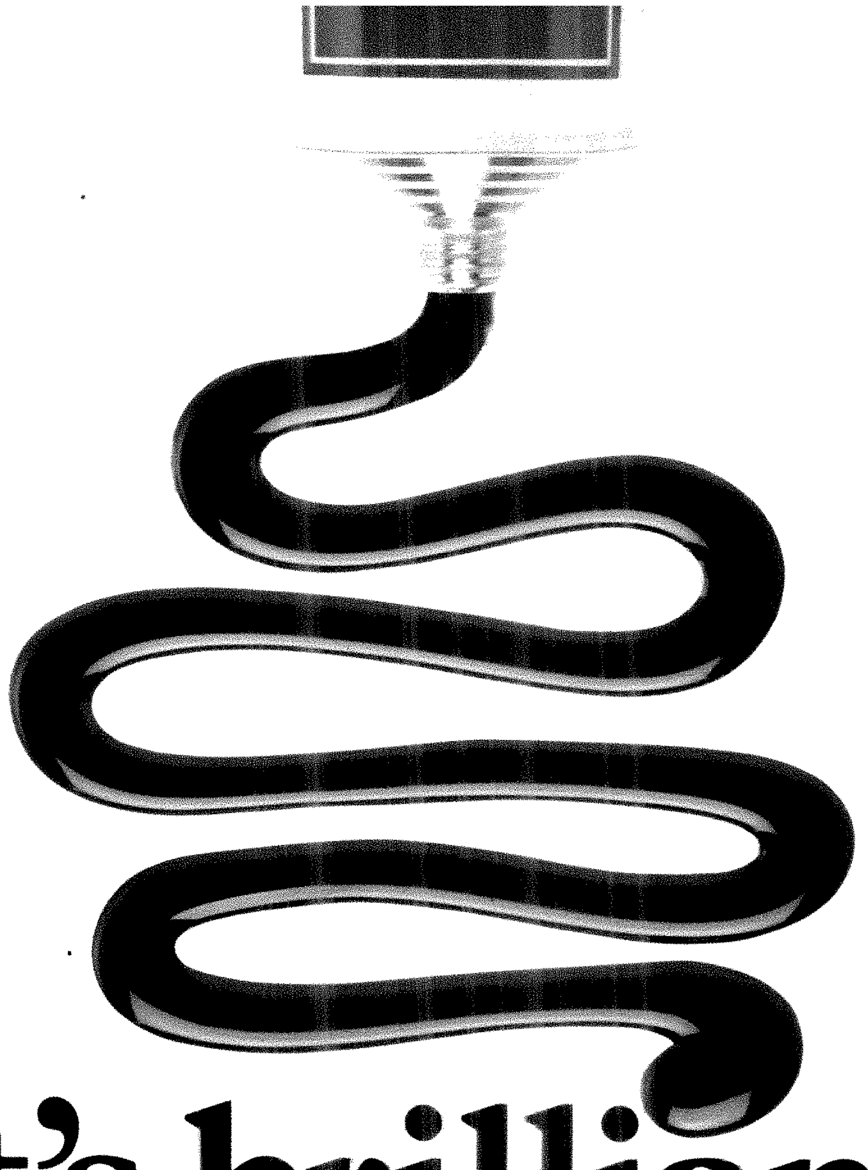
Leaders in topical steroid therapy



It's brilliant' For leg ulcers

Developed as a *specific* therapy for leg ulcers, Variclene — containing brilliant green in an antiseptic combination — dries the ulcer site, combats infection and aids the healing process.

That's why it's brilliant... for leg ulcers.

Further information is available from:
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Prescribing Information

Use: An aid in the topical treatment of venous and other types of skin ulcers.

Directions: Clean and dry the ulcer site, apply and smooth the Variclene gel over the affected area. Cover with a sterile dressing or pad and, where appropriate, apply compression bandaging. Repeat as required at intervals of not more than seven days.

Contra-indications, Warnings, etc: Avoid contact with clothing and linen. Occasionally, transient stinging on application may occur. In cases of arterial or extensive, painful venous ulceration, this may be prolonged, when treatment should be discontinued until greater tolerance has been achieved. Keep away from the eyes.

Presentation: 50g tube **Product Licence:** 0173/0015 (PA 278/10/1)

Variclene®

Green aqueous gel containing 0.5% w/w Brilliant Green BP, 0.5% w/w Lactic Acid B

RESULTS

A black-brown deposit was observed on the epidermal basement membrane on semithin sections, contrasting with the slight background staining (Fig. 1). Serial sections observed under phase contrast microscopy were performed in order to choose the best area for ultrastructural examination.

In bullous pemphigoid (Fig. 2) C3 deposits were observed in the lamina lucida, but more precisely on the hemidesmosomes and in a thin linear pattern on the basal cytoplasmic

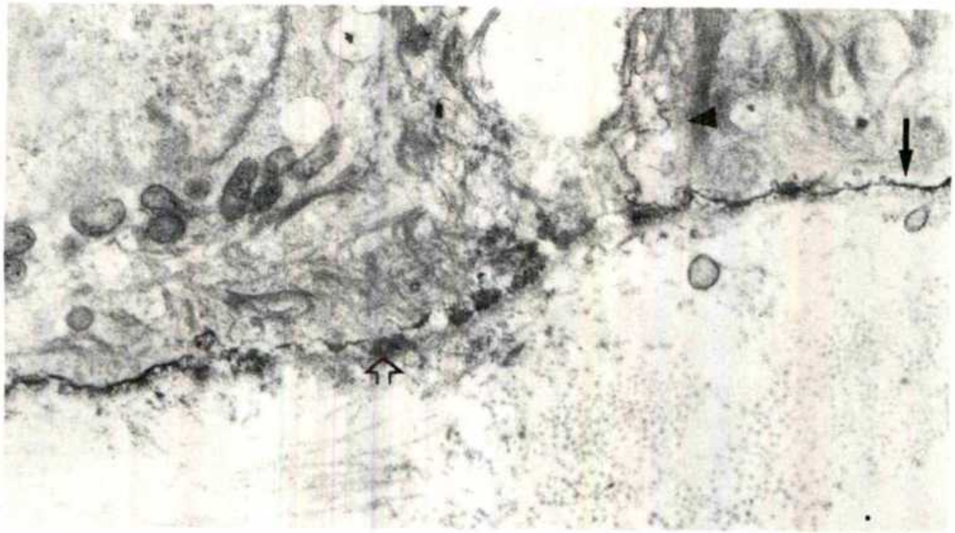


FIGURE 2. Bullous pemphigoid. C3 deposits in the upper part of the lamina lucida (→), on hemidesmosomes (⇒) and on the lateral cytoplasmic membrane of the basal keratinocytes (◄) (× 18,500).

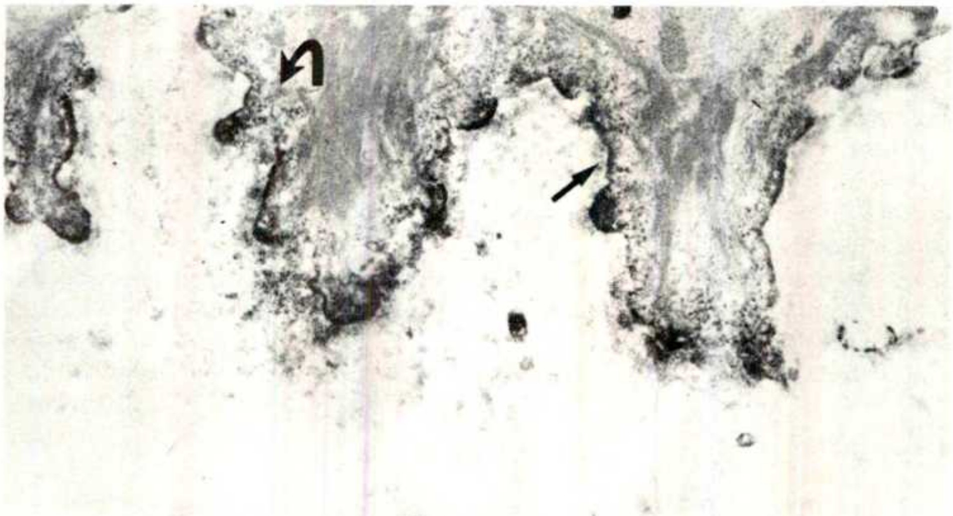
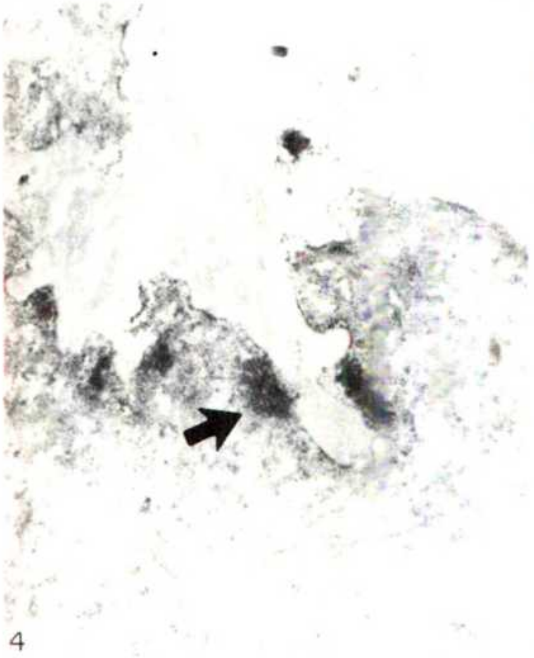


FIGURE 3. Cicatricial pemphigoid. C3 deposits on the basal cytoplasmic membrane (→) and in the cytoplasm (↶) of the basal keratinocytes (× 24,400).



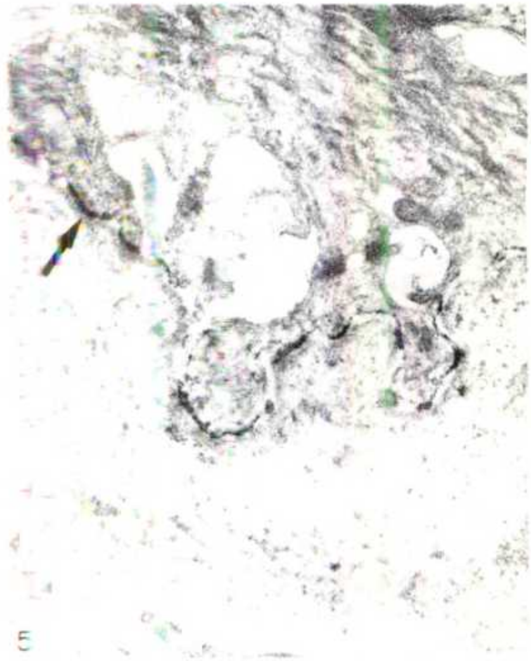


FIGURE 4. Epidermolysis bullosa acquisita. IgG deposits in a nodular pattern beneath and close to the lamina densa (←) (× 17,250).

FIGURE 5. IgA linear disease. IgA deposits in the upper part of lamina densa on hemidesmosomes (→) (× 19,700).

membrane of the keratocytes. C3 deposits could be also seen on the lateral surface of the cytoplasmic membrane of basal keratocytes.

In cicatricial pemphigoid (Fig. 3) the C3 deposits were observed on the cytoplasmic membrane of basal keratocytes. Furthermore intracytoplasmic staining of special interest, close to the basal cytoplasmic membrane in a 'clump' pattern, could be observed.

In one suspected case of EBA (Fig. 4) heavy spots of IgG deposition were observed beneath but close to the lamina densa in a nodular pattern. A control without Ig did not show amorphous material below the basal lamina anchoring fibril zone.

Linear IgA diseases seem to be quite a heterogeneous group under the electron microscope. In one patient (Fig. 5) discontinuous IgA deposits were observed in the lamina lucida on hemidesmosomes as in bullous pemphigoid. In three patients (Fig. 6), IgA deposits were seen beneath the lamina densa in a reticular pattern and also clearly on the basal cytoplasmic membrane of keratocytes.

All the controls gave negative results.

DISCUSSION

As immuno-electron microscopy is becoming more readily available in dermatology, it seems suitable to propose a simple technique allowing good morphological results, good Ig tissue penetration and easy manipulation. The use of skin slices of 0·7 mm thickness proposed in this

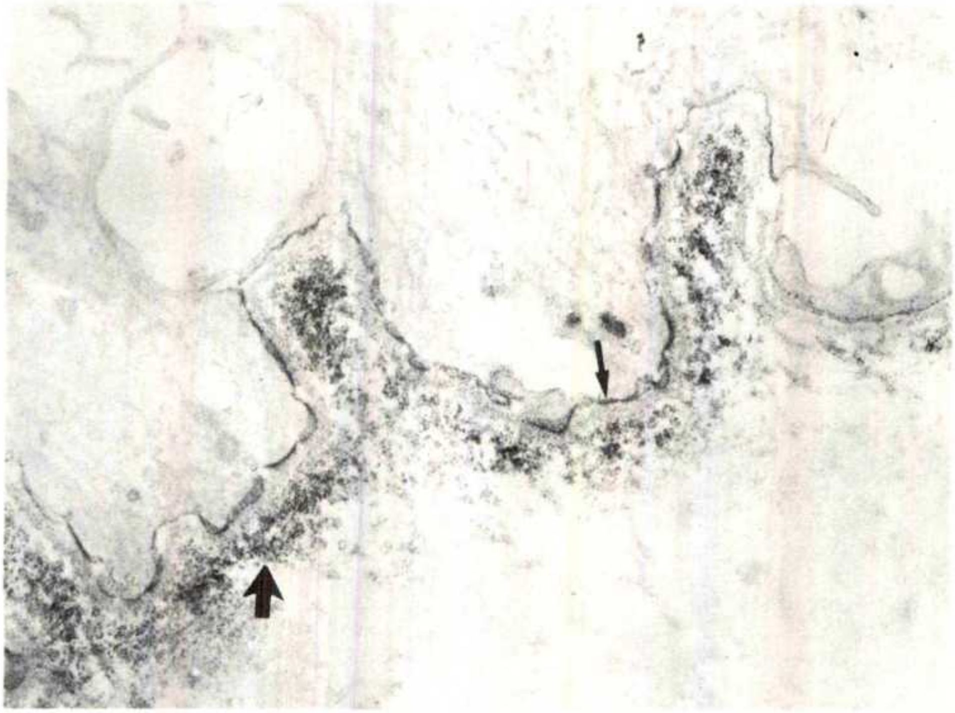


FIGURE 6. IgA linear disease. IgA deposits in a reticular pattern beneath the lamina densa (➞) and on the basal cytoplasmic membrane of the basal keratinocytes (→) (× 22,600).

technique has been already shown as allowing easy manipulation and a good penetration of fixatives and reagents (Dubertret *et al.*, 1980). Washing the fresh skin slices in a large volume of Hank's medium is a key point of the technique because it allows the elimination of extracellular immunoglobulins (Brandtzaeg, 1974). If the washing is insufficient, these extracellular immunoglobulins would react with the anti-Ig labelled antibody, making impossible the penetration of labelled antibody up to the specific auto-immune deposits. The buffered 4·5% formaldehyde solution used as described makes good morphological preservation possible during the long incubation time as well as the preservation of the antigenicity of basement membrane immunoglobulin deposits.

The negativity of our controls demonstrated the specificity of the observed staining.

Most patterns observed with this technique were similar to those observed in previous work i.e. in the pemphigoid group of disorders (PB and CP), localization of IgG deposits in the lamina lucida and association with hemidesmosomes (Albini *et al.*, 1979; Holubar *et al.*, 1975; Schmidt-Ulrich *et al.*, 1975; Nieboer *et al.*, 1980); in linear IgA diseases, heterogenous patterns of IgA deposits and heterogenous antigenic determinants, above the lamina densa in the laminar subtype, or also above and below (Leonard *et al.*, 1982; Dabrowski *et al.*, 1978;Yaoito & Katz, 1976, 1977; Yamasaki *et al.*, 1982; Haffenden *et al.*, 1983; Katz & Strober, 1978).

Moreover the quality of the morphological results makes it possible to give a very precise localization of Ig deposits. Thus in PB we see clearly and directly the close association of PB antigen with the cytoplasmic membrane of the basal keratocytes, previously demonstrated by indirect procedures on junctional blisters (Stanley *et al.*, 1982). The same localization of IgA deposits at the upper part of the lamina lucida suggests a similar biochemical nature of antigenic determinant in PB and laminar linear IgA disease.

The interesting intracytoplasmic C3 deposits in cicatricial pemphigoid suggest an alteration in the basal cytoplasmic membrane, allowing C3 penetration into the cell.

In the patient with clinical features of EBA, the IgG deposit close to the lamina densa and unrelated to any deposit of amorphous material, seems different from those described in previous reports (Nieboer *et al.*, 1980; Wechsler *et al.*, 1982; Yaoita *et al.*, 1981). Similar bullous disorders have been recently very briefly reported (Briggaman *et al.*, 1983). Further studies are necessary to interpret this group of disorders with sublamina-densa IgG deposits.

In conclusion, the routine immuno-electron microscopic technique described here should allow easier and more precise correlation between the clinical and the immunological events in subepidermal auto-immune bullous diseases.

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Indirect immunofluorescence microscopy of lichen planus

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SUMMARY

Indirect immunofluorescence microscopy using serum and lesional skin revealed a lichen planus specific antigen (LPSA) in 80% of patients with lichen planus. This antigen is found only in the stratum granulosum and stratum spinosum. It was demonstrated in skin lesions in Caucasians and Negroes in South Africa and the U.S.A. but was not found in the skin of normal people or patients with other dermatoses. Indirect immunofluorescence should prove useful in distinguishing atypical forms of lichen planus from other dermatoses.

Lichen planus sometimes poses problems in diagnosis, particularly when it is ulcerated, atrophic, hypertrophic, follicular or bullous. Direct immunofluorescence microscopy of lichen planus lesions reveals deposits of immunoglobulin and fibrin within cytoid bodies and at the dermo-epidermal junction in a large percentage of cases studied (Abell *et al.*, 1975; Laskaris, Skavonou & Angelopoulos, 1982) but deposition of immunoglobulin and fibrin also occurs in certain other dermatoses (Laskaris *et al.*, 1982). Thus direct immunofluorescence does not appear to detect specific markers for lichen planus.

We recently demonstrated a lichen planus specific antigen (LPSA) by indirect immunofluorescence using patient serum and autologous tissue or allogeneic lesional tissue from other patients with lichen planus (Olsen *et al.*, 1983). The LPSA occurred in the stratum spinosum and stratum granulosum but not in the stratum corneum, basal layer, basement membrane or dermis. The objectives of the present study were to determine whether LPSA was a marker unique to lichen planus in patients of different racial origins and to assess the feasibility of employing the indirect immunofluorescence assay to differentiate lichen planus from other dermatoses.

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METHODS

Skin biopsies and serum samples from patients with lichen planus were collected from nineteen black patients seen at Ga-Rankuwa Hospital Dermatology Outpatient Clinic and six patients at the Ohio State University Dermatology Outpatient Clinic.

Of the nineteen black South African patients, fourteen were female and five male. Their ages ranged from 11 to 60 (median 30 years) and the duration of the disease from 1 month to 10 years (median 5 months). Two of the patients, a male aged 18 years and a female aged 20 years, had atrophic lichen planus of the hands and feet which had been present with intermittent stages of activity for 8 and 10 years, respectively. In one patient the lichen planus was confined to the vermilion of the lower lip. In the remaining sixteen patients there were varying numbers of active lesions on the limbs, trunk, neck and face. The oral cavity was involved in only two of the nineteen patients.

Of the six American patients, there were five females and one male. One of the female patients was black. Their ages ranged from 39 to 67 (median 4[illegible] years) and the duration of disease from 1 month to 12 years (median 3 months). All patients had active lesions and were receiving no systemic corticosteroids at the time of specimen collection. The oral cavity was involved in three of the six patients.

Skin biopsies and serum samples were taken from eight normal black and three normal white donors. Table 1 lists the dermatoses other than lichen planus used as a control group. The diagnosis was confirmed in each patient by examination of haematoxylin and eosin stained

TABLE 1. Control group dermatoses other than lichen planus

Diagnosis	No. of cases
Lupus erythematosus[a]	10
Eczema	7
Psoriasis	3
Pityriasis rubra pilaris	2
Pityriasis rotunda	2
Pityriasis rosea	1
Pityriasis lichenoides	1
Pemphigus	1
Pemphigoid	1
Dermatomyositis	1
Molluscum contagiosum	1
Excoriations	1
Intradermal naevus	1
Acrochordon	1
Morphoea	1
Dermatofibroma	1
Solar lentigo	1
	36

[a] Seven DLE, three SLE.

biopsies. In addition, a biopsy of normal skin was taken from twenty of the twenty-five patients with lichen planus. [Eight patients with lichen planus, eight with other dermatoses and eight normal volunteers investigated in the previous study (Olsen *et al.*, 1983) were included here.]

Sixty-two sera taken from a random epidemiological survey were tested on cryostat sections of lichen planus lesions.

Methods for tissue collection and cryostat sectioning were described earlier (Olsen *et al.*, 1983).

Immunofluorescence. The reagents used in the direct and indirect immunofluorescence antibody methods were as described previously (Olsen *et al.*, 1983). The immunofluorescence reagent used in both direct and indirect immunofluorescence assays was polyvalent goat anti-human immunoglobulin obtained from the Wellcome Reagents Limited, Beckenham, England. Indirect immunofluorescence was performed according to methods described by Beutner, Nisegold & Kumar (1979).

RESULTS

Indirect immunofluorescence microscopy revealed LPSA in the lesional skin in 80% (20/25) of lichen planus patients studied (Table 2). In the twenty positive cases, LPSA was evident only in the stratum spinosum and stratum granulosum (Fig. 1). No specific fluorescence was noted in the dermis, basal layer or stratum corneum. The immunofluorescence staining pattern described above was negative in thirty-six patients with other dermatoses and eleven normal

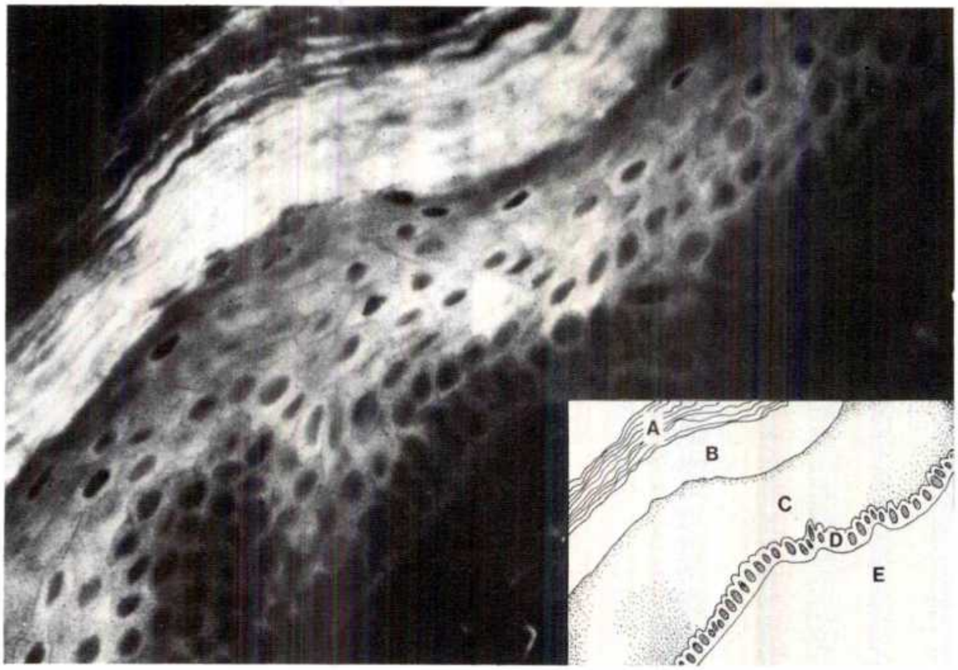

FIGURE 1. Indirect immunofluorescence microscopy of lichen planus with patient's autologous serum revealed lichen planus specific antigen in the stratum granulosum (B) and stratum spinosum (C). No significant fluorescence was observed in the stratum corneum (A), basal layer (D) or dermis (E).

TABLE 2. Reaction of serum from lichen planus patients on tissue sections of lichen planus and other dermatoses

Skin tissues[a]	Number tested	Positive immunofluorescence[b] Indirect	Direct
Lichen planus	25	20	0[c]
Non-lichen planus dermatoses	36	0	nt
Normal skin[d]	11	0	nt

[a] Cryostat sections.

[b] The criterion for indirect and direct immunofluorescence was a specific fluorescence of the stratum granulosum and stratum spinosum as illustrated in figure 1.

[c] Zero out of fourteen tested.

[d] Normal skin from healthy donors.

nt = not tested.

individuals with no history of skin disease (Table 2). Direct immunofluorescence did not reveal a specific fluorescence pattern in the stratum spinosum or stratum granulosum in biopsies of lichen planus.

Specificity studies

Table 3 summarizes the results from studies designed to evaluate the specificity of the indirect immunofluorescence assay for LPSA. The percentage of positives for LPSA in lichen planus lesional skin in patients studied in the United States was similar to that in South Africa. In

TABLE 3. Characterization of the lichen planus specific antigen (LPSA) as a marker for lichen planus (LP)

Tests performed	Samples tested United States[a]	South Africa[b]	Total tested
Incidence of LPSA in lesional skin	5/6[c]	15/19	20/25
LP serum (LPSA-positive) tested on allogeneic LP lesional skin	4/4	6/6	10/10
LP serum (LPSA-positive) tested on autologous normal skin	1/1	2/19	3/20
LP serum (LPSA-positive) tested on skin from healthy donors	0/3	0/8	0/11
Serum from healthy donors tested on LP lesional skin	0/3	4/59	4/62

[a] Five out of six patients were white.

[b] All patients were black.

[c] Number positive/total number tested.

addition, LPSA was detected by LPSA-positive patient serum in various allogeneic lichen planus lesional tissues. Serum positive for LPSA, however, failed to detect antigen in those cases where LPSA was not evident in the autologous reaction (i.e. patient serum tested on own lesional tissue) (Table 3).

Five patients with lichen planus did not have detectable LPSA in lesions. One of these patients was white and four were black. Of the latter, two had lichen planus of the acral atrophic type. Serum from these patients also did not react with lesional skin from other patients which had been shown to be LPSA-positive. In one of these patients, an elderly black woman with diabetes mellitus who was being treated with prednisone, biopsies of active lesions were taken 7 months apart and each was LPSA-negative.

Three out of twenty patients with lichen planus tested exhibited LPSA in biopsies taken from what appeared to be normal skin. In two cases the normal skin biopsy was taken near to the site of the biopsied lesion. The third patient appeared to be in an acute eruptive phase of the disease.

No LPSA was detected in skin from healthy donors. Four of sixty-two sera taken for a random epidemiological survey had what appeared to be antibody to LPSA when tested on lichen planus lesions. One of these four donors had a history of a pruritic eruption suggestive of eczema.

DISCUSSION

We previously described a lichen planus specific antigen (LPSA) in the stratum granulosum and stratum spinosum in lesions from patients with lichen planus (Olsen *et al.*, 1983). The results of the present study demonstrate that LPSA is a common marker for lichen planus in patients of diverse racial origins and that the serological demonstration of LPSA distinguishes lichen planus from other dermatoses. The method used to demonstrate LPSA was an indirect immunofluorescence assay in which the patients' sera were reacted with their own lesions. In our experience, the direct immunofluorescence assay showed an occasional lichen planus biopsy with deposition of immunoglobulin at the dermo-epidermal junction and within cytoid bodies. The direct assay did not demonstrate any fluorescence in the stratum granulosum or stratum spinosum as seen in the indirect method.

Previous reports of immunofluorescence studies in lichen planus have dealt only with direct immunofluorescence assays (Baart De La Faille-Kuyper & Baart De La Faille, 1974; Abell *et al.*, 1975; Jablonska, Chorzelski & Blaszczyk, 1976; Laskaris *et al.*, 1982). These studies revealed a high frequency of immunoglobulin, complement and fibrin deposition in the dermo-epidermal zone and in cytoid bodies but these markers are not specific for lichen planus. In particular, direct immunofluorescence microscopy may not differentiate between lichen planus and lupus erythematosus (Michel *et al.*, 1970). Both diseases show a band-like deposition of immunoglobulins at the dermo-epidermal junction and in cytoid bodies (Abell *et al.*, 1975). We tested ten patients with lupus erythematosus, and none exhibited a specific indirect immunofluorescence for LPSA. The indirect immunofluorescence method should therefore prove valuable in difficult diagnostic problems found in atypical forms of lupus erythematosus, particularly where the inflammatory infiltrate is superficial and band-like. Patients with chronic graft-versus-host reaction (GVHR) may present with violaceous lichenoid papules on distal extremities (Farmer & Hood, 1983) and mucosal lichenoid keratoses (Rodu & Gockerman, 1983) that closely resemble lichen planus. Interestingly, indirect immunofluorescence of serum from a 17-year-old white male with lichenoid chronic GVHR 5 months after marrow transplantation was negative for LPSA using LPSA-positive allogeneic LP lesional skin as substrate. The patient was receiving systemic corticosteroids and immunosuppressive therapy, and a skin biopsy could not be obtained because of profound thrombocytopenia. The immunological relationship of lichen planus and lichenoid chronic GVHR needs further study.

Five out of twenty-five of our patients with lichen planus exhibited no LPSA in their lesions. Moreover, no antibody to LPSA could be detected by reacting their serum with known LPSA-positive skin sections. Two of these LPSA-negative lichen planus patients were in a late atrophic stage and it may be assumed that the antigen and antibody had disappeared. Both of these patients have been first diagnosed years before when they were in an active stage. One LPSA-negative patient had active lesions but was being treated with systemic corticosteroids. However, of the LPSA-positive patients, three were also being treated with corticosteroid. One LPSA-negative patient was not treated with corticosteroids and had active lesions. Corticosteroid treatment was probably not responsible for the negative findings. These negative findings suggest that LPSA is not produced in all lichen planus lesions or that LPSA is absent in the lesion during some stages of the disease, or that it may not always be detectable by this method.

The finding of LPSA in apparently normal skin of three patients with lichen planus may be due to subclinical involvement of the so-called 'normal' skin.

The biochemical relationship of LPSA to epidermal cytoplasmic antigens (Burnham, 1977) and stratum corneum antigen (Krogh, 1970) is unknown. The epidermal cytoplasmic antigens include the upper epidermal cytoplasmic (UEC) and general epidermal cytoplasmic (GEC) antigens. These antigens are detected by indirect immunofluorescence employing normal skin sections and serum from normal people. The incidence of antibody in normal people to UEC and GEC is 21% and 8%, respectively (Burnham, 1977). Antibody to the stratum corneum antigen (SCAg) has been detected by haemagglutination methods (Krogh, 1970) and by indirect immunofluorescence (Beutner *et al.*, 1982). Both techniques reveal that antibodies to SCAg are present in all human sera (Beutner *et al.*, 1982). Moreover SCAg, like UEC and GEC, appears to be a component of normal skin (Binder, Beutner & Jablonska, 1982). These antigens differ from LPSA in that they are found in normal skin and their corresponding antibodies are detected in relatively high incidences in people with no known dermatoses. In addition, the LPSA is found exclusively in the stratum granulosum and stratum spinosum. The SCAg occurs in the stratum granulosum and stratum corneum but is absent in the stratum spinosum (Beutner *et al.*, 1982). The UEC antigen is found in the middle and upper layers of the epidermis but not in the basal layer, while GEC is demonstrated in all epidermal cells (Burnham, 1977). It is evident that LPSA is unrelated to the above described epidermal antigens and that LPSA is not a normal skin component.

Though LPSA is associated with lichen planus, it may be a secondary phenomenon, and further research is needed to determine its role in the pathogenesis of lichen planus.

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Effect of retinoic acid and low calcium conditions on surface glycoconjugates defined by differential lectin labelling in mouse epidermal cell culture

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SUMMARY

The appearance of cell surface glycoconjugates (detected by fluorescein-isothiocyanate-conjugated lectins and bullous pemphigoid antibody) was serially examined in mouse epidermal cell cultures treated with *trans*-retinoic acid and aromatic retinoic acid (etretinate) and in cultures maintained under low calcium conditions. The changes in lectin staining occurred in concert with the process of differentiation as assessed by cell morphology and colony growth characteristics, and they correlated with the patterns observed in whole mouse skin.

The keratocyte cultures treated with retinoic acid showed delayed and reduced differentiation and stratification, and this was associated with markedly reduced binding of lectins specific for *N*-acetyl-glucosamine and fucose. The low calcium concentration produced similar changes. Thus, the loss of surface glycoconjugates in the epidermal cell culture system was not specific for either retinoic acid or low calcium, but correlated with the degree of cell differentiation.

Retinoids profoundly influence proliferation and differentiation of epithelial cells. They inhibit the growth of some epidermal tumours and the epidermis in certain hyperproliferative states (Mahrle & Berger, 1978; Mahrle, Meyer-Hamme & Ippen, 1983; Stuttgen, Ippen & Mahrle, 1977), but they stimulate proliferation of normal epidermis (Pullman, Lennartz & Steigleder, 1975). They also loosen the squames of the stratum corneum and reduce the cohesion of keratocytes via altered cell junctions and widened intercellular spaces (Brown & Habowsky, 1979; Matler & Bollag, 1977; Yuspa & Harris, 1974; Hardy, Sweeny & Bellows, 1978). These changes are accompanied by reduced formation of keratin (Tsambaos, Mahrle & Orfanos, 1980) and increased production of glycoconjugates (De Luca & Yuspa, 1974).

Although the mechanism by which retinoids alter epidermal differentiation is not fully understood, changes in cell membrane glycoconjugates may contribute to these effects.

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ferentiation of keratocytes is accompanied by predictable changes in the content of exposed bohydrate moieties on the cell surface as demonstrated by binding of lectins with specific bohydrate affinities (Brabec, Peters & Bernstein, 1980; Nemanic & Elias, 1979). Addition of erent sugars to the surface glycoconjugates during maturation of the cell may account for se changes (Zieske & Bernstein, 1982). This view is consistent with the fact that glycosyl asferase activity is linked to the differentiation of rat gut epithelium (Kim *et al.*, 1975). De a has proposed that vitamin A acts as a carrier of glycosyl moieties in the synthesis of oconjugates (De Luca, 1977). It is thus of interest that the epidermis of mice treated with a oid displays altered patterns of carbohydrates in cell surface glycoconjugates as demon- ed by lectin binding (Nemanic, Fritsch & Elias, 1982).

pidermal cell cultures have been extensively used to examine factors which control liferation and differentiation and which affect the structure and configuration of keratocytes. h the exception of bullous pemphigoid (BP)-antibody staining of basal cells (Diaz & celo, 1978) and lectin-staining of guinea-pig epidermal cell cultures (Mahrle, Wilkinson & er, 1981), surface glycoconjugates have not been extensively examined in epidermal cell tures. The present studies were undertaken to examine the surface glycoconjugates in mary keratocyte cultures in relation to differentiation. They were designed to test the validity cell cultures as a model of *in vivo* modulation in surface glycoconjugates. The presence of cific sugars was assessed by binding of lectins and BP antibodies in normally differentiating tures. The specificity of the changing patterns of lectin binding in relation to maturation of atocytes was assessed in cultures in which differentiation was inhibited by exposure to low cium concentrations or retinoids.

METHODS

ces of growth media and chemicals

e reagents and their sources were as follows: trypsin, Eagle's minimal essential medium EM), fetal calf serum (FCS) and antibiotic-antimycotic solution were from GIBCO, Grand and, N.Y.; Ficoll 400, ethyleneglycol-bis-(β amino-ethyl ether), and *N*-*N*′-tetra-acetic acid TA) were from Sigma Chemical Company, St Louis, MO.

P antibody was demonstrated using serum from a patient with pemphigoid having a titre of ater than 1:40. FITC goat anti-human IgG serum was from Miles Laboratory.

The FITC lectins were obtained from the following sources: Ricinus communis agglutinin I lysciences, Inc., Warrington, PA), Ulex, soybean, wheat germ agglutinin, and concanavalin (Miles Laboratory, Elkhart, IN). Polyclonal anti-keratin antiserum was supplied by Dr T. Sun. Its preparation and properties have been previously described (Sun & Green, 1978).

Trans-retinoic acid (tRA) came from Sigma Chemical Company, St Louis, MO and aromatic noic acid (aRA) (etretinate) was supplied by Hoffman-LaRoche, Nutley, NJ.

l culture procedures

n of newborn Balb/c mice was prepared as described previously (Yuspa, 1978). The atocyte cell suspension was further purified by discontinuous Ficoll gradient centrifugation. is yielded a population of basal cells to suspend in MEM (Ca^{2+}; 1·27 mM) supplemented with % FCS, 50 μg/ml gentamicin, 5 μg/ml mycostatin, and 100 μg/ml carbenicillin. Cells were ted at a density of $3 \times 10^6/cm^2$ in 1·0 ml placed on to a 22 mm^2 glass coverslip and grown at C, 5% CO_2, pH 7·0–7·2. These optimal growth conditions formed the basis for the control ies. In all experiments, the cells were allowed to attach for the first 24 h in culture medium without additions or deletions and then washed twice with phosphate-buffered saline (PBS). The media were changed every other day. At the time of cell plating, cell viability, as determined by the Trypan Blue exclusion test, was 90% of the basal cells.

Retinoids, either tRA or aRA, were added to the cultures in 2 μl of ethanol to achieve a final concentration of $1{\cdot}7 \times 10^{-5}$M, $1{\cdot}7 \times 10^{-6}$M, or $1{\cdot}7 \times 10^{-7}$M. Retinoids, which were handled in subdued light, were added to cultures 24 h after plating and with every medium change. All cultures supplemented with retinoids were kept in the dark.

In another group of experiments, the cultures were plated under the same conditions as the controls, but 24 h after plating they were washed twice with PBS and then maintained at low calcium conditions of 0·15 mM. This medium was prepared by removing the excess calcium from the control medium with 0·318 mM EGTA. In all experiments, paired control and treated cultures were harvested daily.

Cellular morphology and colony growth characteristics were monitored on a phase contrast microscope, Biostar I.

Immunofluorescent (IF) techniques. IF with BP serum and FITC lectins.

Four-micron sections of neonate Balb/c mouse leg cut on a cryostat were used as substrates for control testing of the indirect IF staining using BP serum and FITC lectins (Beutner *et al.*, 1973).

Treated and control epidermal monolayers grown on glass coverslips were fixed in acetone at −20°C for 30 sec, rinsed with PBS and stored in a freezer at −70°C. In preliminary experiments, cells fixed in alcohol exhibited no difference in binding patterns from those fixed in acetone. Thus acetone was used because it proved to be a better fixative.

The coverslips were incubated for 30 min in a moisture chamber at room temperature with BP serum. After washing with PBS, the coverslips were then incubated with FITC goat anti-human IgG serum. Blocking experiments were performed by pre-incubating the epidermal monolayers with unlabelled goat anti-human IgG serum and then performing the indirect IF procedure.

Sections were incubated for 30 min in a moisture chamber at room temperature with FITC-labelled lectins in 0·5 M PBS, pH 7·3, rinsed in the buffer and examined with a Zeiss I photomicroscope at 490 nm using a 530 nm barrier filter. Concentrations of the various FITC lectins were used as follows: concanavalin A at 75 μg/ml, Ricinus communis I at 100 μg/ml, soybean agglutinin at 75–100 μg/ml, Ulex agglutinin at 125/150 μg/ml, wheat germ agglutinin at 50–100 μg/ml.

A solution of the same FITC lectin concentration was applied to sections pre-incubated for 30 min at 20°C with 0·2 M of competing sugar hapten and then rinsed with PBS (Table 1). Sections were also incubated with FITC alone. Three representative coverslips were examined blind for each datum point and an average result was recorded. The intensity of fluorescence was graded semi-quantitatively from 0 to 4:4 represented staining of more than 80% of the cells on the coverslip, whose total surface was examined in 225 fields at 80 × magnification, 3 was 60–80% of cells, 2 was 40–60%, 1 was 20–40%, trace was 0–20% and 0 no cells stained. Variation in intensity of fluorescence was never more than one point on the grading system between triplicate samples. Photographs were taken with Ektachrome 400.

The coverslips were sequentially incubated with the following reagents for 45 min each at 37°C and washed with PBS after each incubation: 20 μl of polyclonal anti-keratin antiserum and 20 μl of FITC goat anti-rabbit IgG (diluted 1:16 with PBS). Coverslips were mounted with Gelvatol®. The optimal dilution of the polyclonal anti-keratin antiserum was determined for

TABLE 1. Lectin with saccharide specificity, binding inhibitor (Freeman, Lotan & Kim, 1980) and area of epidermis labelled in whole rat skin (Brabec *et al.*, 1980) and the neonate mouse

Lectin	Saccharide specificity	Binding inhibitor	Epidermal labelling Whole rat skin	 Neonate mouse
Concanavalin A	α-D-mannosyl α-D-glucosyl	α-methyl-mannoside	All layers except stratum corneum	
Ricinus communis agg.	β-D-galactosyl	Galactose		Basal
Soybean agg.	*N*-acetyl-galactosamine	Galactose	Mid-epidermis	Weak basal Lower spinous
Ulex agg.	α-L-fucose	α-L-fucose	Spinous	Upper spinous Granular
Wheat germ agg.	*N*-acetyl-glucosamine	*N*-acetyl-glucosamine	Stratum corneum	Upper granular Stratum corneum

each experiment on mouse epidermis and was 1:50. A pre-immune serum control was negative in all instances.

RESULTS

Growth characteristics

The epidermal cells covered about 80% of the surface of the glass coverslip in 2 days. By day 3 or 4, a complete monolayer had formed and the uppermost cells were polygonal with a large nucleus, and a limited amount of cell stacking was present. Uniform stacking of the culture was present by day 6 to 8. The culture was mostly detached from the coverslip by 7 days and completely detached by 10 days.

When added after 24 h, both tRA and aRA at 10^{-7} M concentration and ethanol alone failed to modify the patterns of cell growth. Cultures treated with tRA or aRA at 10^{-5} M concentration failed to survive. When tRA or aRA at 10^{-6} M concentration was added to the media after the first 24 h (day 0), the cultures became confluent 3 days after attachment (day 3) but the cells remained round and uniform stratification was suppressed. There were only occasional areas of stratified cells at day 5 and the culture remained attached to the coverslips beyond 10 days. tRA or aRA at 10^{-6} M added 4 days after plating of the cell suspension did not affect patterns of colony growth or cell surface lectins.

Cultures grown under low calcium conditions formed a monolayer in 3 days, at which time a few peaks of stratifying cells were evident with a small number of angular cells. After these angular cells were shed by 3 days, no further ones were seen in the culture. The confluent monolayer maintained itself without stratification from day 4 until day 11, when the experiment was halted, and it consisted of cells with uniform ellipsoid shapes.

IF

In whole neonate mouse skin BP antibody was localized to the basal layer of cells. Ric. comm. I strongly stained individual basal cells of whole mouse skin and some lower spinous cells. Soybean agglutinin stained the individual cells of the basal layer less intensely than the lower spinous layer cells of whole mouse skin and failed to stain the granular layer or the upper spinous cells. Whole mouse upper spinous and granular layers, but not basal cells, stained very intensely with Ulex agglutinin. FITC-wheat germ agglutinin stained the granular layer and stratum

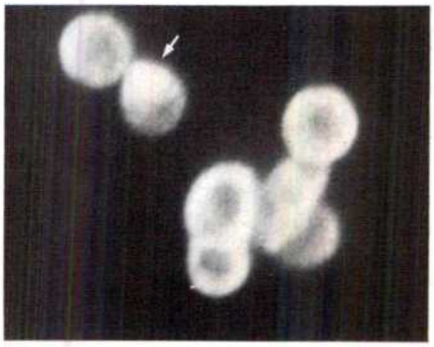

FIGURE 1. Strong rim pattern staining of cells of the pellet with BP antibody. One cell (arrow) has an irregular spot (original magnification × 320).

corneum of whole mouse skin. All layers except the stratum corneum stained with concanavalin A and all layers stained with polyclonal antikeratin serum.

Ninety per cent of the cells of the pellet obtained after Ficoll discontinous gradient centrifugation stained with BP antibody. Some cells stained with BP antibody with fluorescence only at one pole as an irregular spot or rim (Fig. 1). Similar staining has been seen in guinea-pig epidermal cell suspension (Stanley *et al.*, 1980). The cells of the pellet stained intensely in a complete rim pattern with Ric. comm. I and weakly with soybean agglutinin, thus correlating well with patterns of basal cells in whole epidermis. There was no staining with Ulex and wheat germ agglutinin.

In control cultures, BP (not shown) and Ric comm. staining in complete rim surface patterns of round cells was present in over 90% of the cells from 24 h after plating (day 0 of Table 2) to day 2. Intensity of staining with Ric comm. and BP was decreased after day 2 as basal cells were obscured by polygonal stacked cells which did not stain with these lectins. Staining with soybean agglutinin was first detected at day 0 (Fig. 2) but steadily increased in polygonal cells until day 5 when it was obscured by the autofluorescence of superimposed more differentiated cells; in areas of less stacking at the periphery soybean agg. staining persisted. Two-day control

TABLE 2. Summary of fluorescence in control cultures. Intensity was graded 0–4 as described above. Results represent mean of three readings for each datum point

	Fluorescence in control cultures (intensity)							
Lectin								
Ric. comm.	4	4	4	3	2	1	0	0
Soybean	tr	2	3	4	4	2	1	0
Ulex	0	0	1	3	4	4	4	3
Wheat germ	0	0	1	1	3	4	4	4
Day	0	1	2	3	4	5	6	7

tr = trace.

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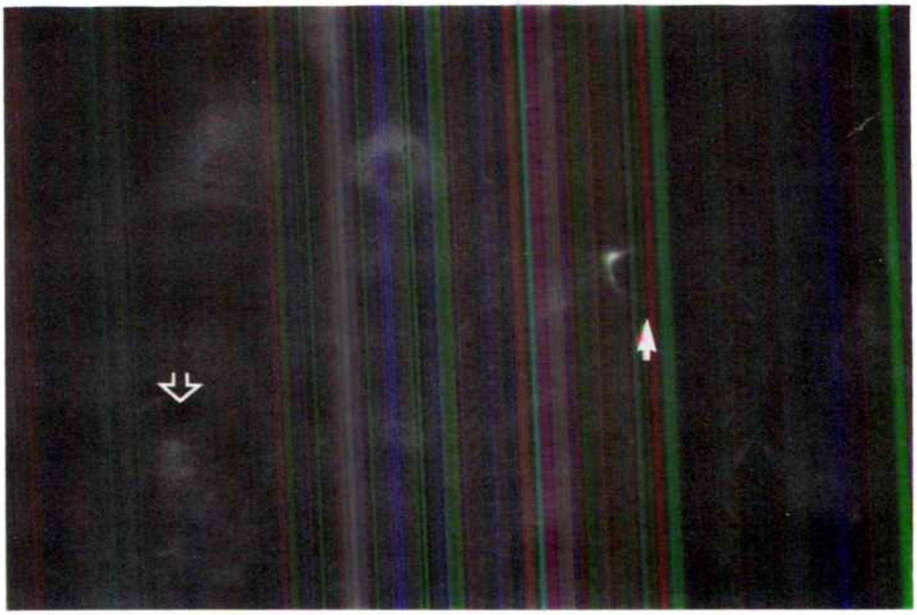

FIGURE 2. Staining of 24 h (day 0) cultures with soybean agg. Non-specific background staining (open arrow) of the field is seen. One round cell exhibits rim pattern staining (solid arrow) in this example of a field designated as trace staining (original magnification × 320).

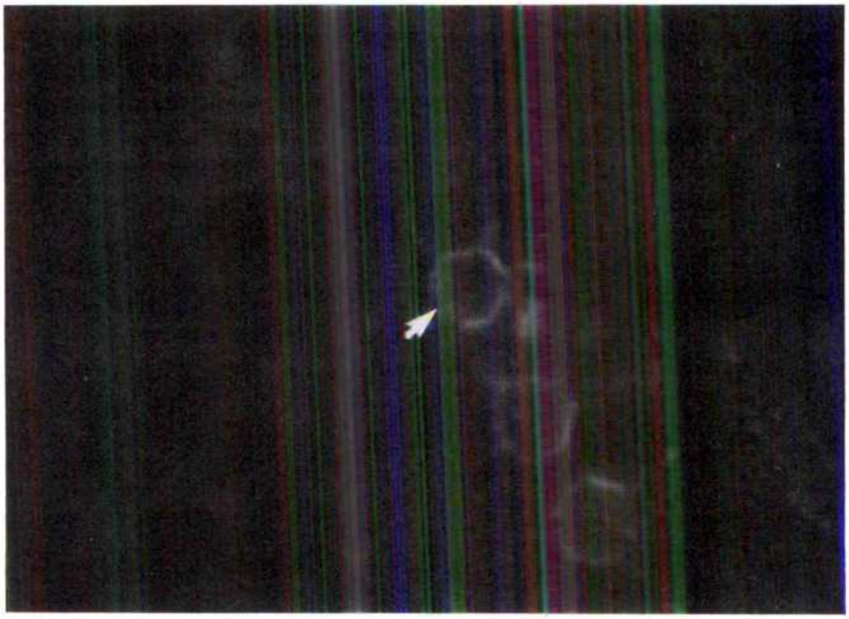

FIGURE 3. In this 2-day control culture, there are intense polygonal patterns of rim staining (arrow) of the cells with Ulex agg. in this field, which is 30% stained and is graded as 1 (original magnification × 320).

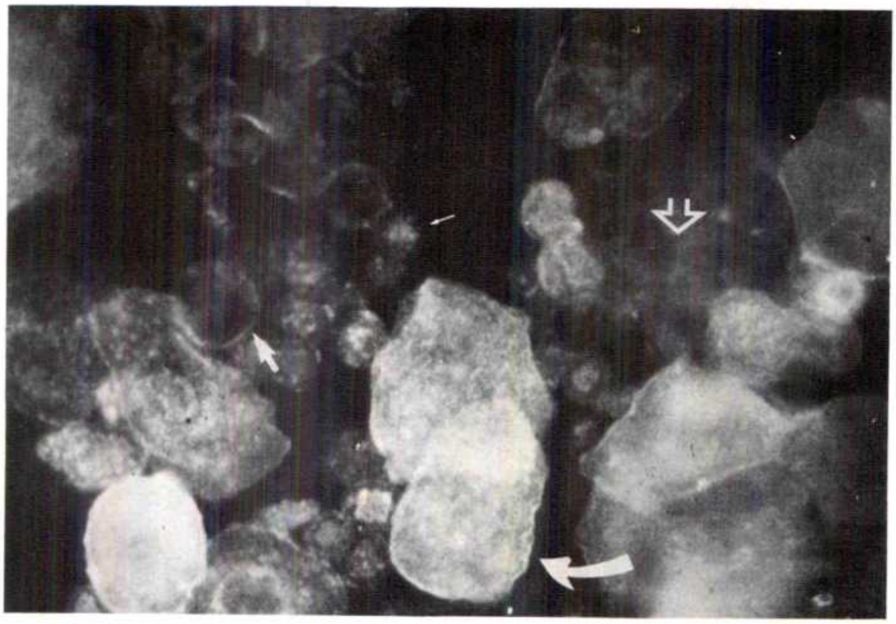

FIGURE 4. A 4-day-old control culture stained with wheat germ agg. exhibits staining of polygonal and round cells (grade 3). While some cells show background staining without fluorescence (open arrow), others show only intracellular fluorescent staining (small, solid arrow). Neither of these patterns of fluorescence is contributory to the 3 rating of this field. Only cells with either rim pattern fluorescent staining (large, solid arrow) or rim pattern and intracellular staining (large, curved arrow) are scored in the rating system (original magnification × 320).

cultures showed staining of polygonal cells with Ulex and wheat germ agg. This increased in intensity until the 5th day (Table 2 and Figs 3 and 4).

Both forms of RA added 24 h after plating preserved staining of round cells with BP and Ric comm. beyond the 8th day of the culture (Fig. 5). A decrease in the intensity of staining was seen at day 5. tRA- and aRA-treated cultures showed two to three cells (trace) per 25 × field, staining with soybean agg. at day 0 as do controls. After 3 days, there was staining with soybean agg.

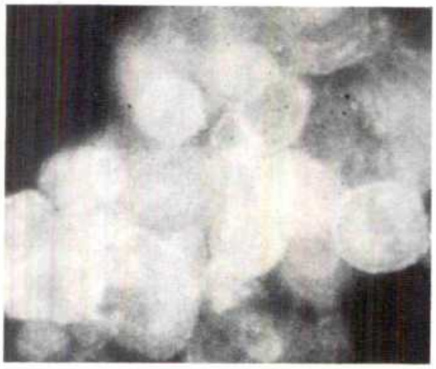

FIGURE 5. 4-day-old retinoic acid-treated culture with round cell staining of every cell in the field with Ric. comm. (grade 4) (original magnification × 320).

TABLE 3. Summary of fluorescence in culture treated with 10^{-6} M *trans*-retinoic acid and aromatic retinoic acid added after 24 h

	Fluorescence in cultures treated with retinoic acid (intensity)							
Lectin								
Ric. comm.	4	4	4	4	4	3	3	2
Soybean	tr	1	tr	1	2	2	2	2
Ulex	0	0	0	0	0	tr	1	1
Wheat germ	0	0	0	0	0	tr	tr	1
Day	0	1	2	3	4	5	6	7
	Add	↑	RA					

(Table 3). Staining with Ulex and wheat germ agg. was never well developed; by day 5 there was minimal reaction (trace–1) in a polygonal pattern.

Both forms of RA added 4 days after plating the cells failed to influence the lectin or BP staining patterns. These cultures stained like control cultures.

Low calcium conditions were initiated after the first 24 h of attachment phase in normal calcium conditions. The influence of the terminal differentiation started during this 24-h period was seen by the staining of 20–40% of the cells with the two lectin markers of differentiated cells, Ulex and wheat germ agglutinin, in polygonal patterns. This corresponds to patterns of differentiating cells seen by phase microscopy described above and confirms a difference between the early effects of low calcium conditions and tRA. By the third day the cultures showed grade 4 uniform round cell staining with Ric. comm. agg. and grade 1 round cell staining with soybean agg. Even though Ulex agg. preferentially stained polygonal cells in control cultures and stained the few polygonal cells which were present in RA-treated cultures at day 5,

TABLE 4. Summary of fluorescence in cultures grown after the first 24 h in medium with a calcium concentration of 0·15 mM

	Fluorescence in low calcium cultures (intensity)							
Lectin								
Ric. comm.	4	4	4	4	4	4	3	3
Soybean	tr	1	tr	1	2	2	2	2
Ulex	0	0	1	0	0	tr	1	1
Wheat germ	0	0	1	0	0	0	0	0
Day	0	1	2	3	4	5	6	7
		↑ low calcium						

it stained round cells of 5, 6, and 7 day cultures maintained under low calcium conditions. These cultures did not stain with wheat germ agg. (Table 4).

Both control cultures and RA-treated cultures showed cells with prominent cytoskeletons with accentuation of the perinuclear wavy bundles by indirect IF with anti-keratin serum.

DISCUSSION

In these studies lectins and BP antibody were used as probes for demonstrating the sequential development of specific cell surface glycoconjugates in relation to morphological evidence of differentiation in primary epidermal cell cultures. The intensity of staining and sharpness of the ends of the range of specificity for cell layers varies with each agent used. As has been shown by others (Brabec *et al.*, 1980; Gommans & van den Hurk, 1982; Nemanic & Elias, 1979; Stanley *et al.*, 1980; Podolsky & Weiser, 1973). BP and Ric. comm. were demonstrated in round (basal) cells. Reaction with BP was uniformly intense and sharply limited to normal basal cells. On the other hand Ric. comm. and soybean agg. overlap in staining of various layers in whole skin and show greater gradation in the intensity of individual cell staining. As the cultures formed monolayers and stratified, the pattern of cell surface glycoconjugates detected by binding of lectins to specific sugar residues changed. The staining of BP antigen and galactose (Ric. comm.) were supplanted by *N*-acetyl-galactosamine (soybean) which was in turn supplanted by fucose (Ulex) and *N*-acetyl-glucosamine (wheat germ). The changes in lectin staining occurred at the same time as the process of differentiation as assessed by cell morphology (round or flattened cells), and colony growth characteristics (monolayer or stratified).

The findings correlated with the pattern in whole adult mouse skin (Table 1) where the various antigens are characteristic of progressively more differentiated cells (Brabec *et al.*, 1980; Nemanic & Elias, 1979; Brysk & Snider, 1982). Thus, irrespective of other characteristics of primary mouse epidermal cell cultures, the cell surface glycoconjugates seem to be similar to those occurring in whole epidermis, just as the cell surface glycoconjugates of human primary cell suspensions (Gommans & van den Hurk, 1981) were similar to those in whole human epidermis (Gommans & van den Hurk, 1982). Arrest or delay of differentiation by either retinoids or calcium deprivation prevented or greatly diminished the orderly appearance of *N*-acetyl-galactosamine, fucose, and *N*-acetyl-glucosamine on the cell surface. This is similar to the decreased binding of lectins specific for the latter two sugars in mouse skin after treatment with retinoids *in vivo* (Nemanic, Fritsch & Elias, 1982) and the loss of galactosamine and galactosyl sugars in guinea-pig epidermal cell cultures treated *in vitro* (Mahrle *et al.*, 1981). Since it is likely that the effects of RA and calcium depletion are mediated by different mechanisms, the data suggest that the distinctive patterns of the carbohydrate moieties of surface glycoconjugates are intrinsic properties relating to differentiation *per se*. Moreover, these studies have shown that the primary keratocyte culture provides a suitable model for investigation of cell surface glycoconjugates.

ACKNOWLEDGMENTS

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Decreased induction of aryl hydrocarbon hydroxylase activity in hyperproliferative hairless mouse epidermis

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SUMMARY

Hairless mice were treated topically with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to induce experimental chloracne. The aryl hydrocarbon hydroxylase (AHH) activity in the epidermis was monitored. TCDD is a potent inducer of AHH activity in normal skin. However, as continued TCDD application induced hyperproliferative and hyperkeratotic changes in the epidermis, the AHH activity decreased. Similar suppression in AHH activity was demonstrated in epidermis made hyperplastic by (a) tape stripping of stratum corneum and (b) repeated application of 50% oleic acid. This suggests that the epidermal AHH response varies with the state of epidermal differentiation. In the hairless mouse, AHH activity in hyperplastic epidermis is lower than that in normal epidermis.

Aryl hydrocarbon hydroxylase (AHH) belongs to a group of inducible mixed-function mono-oxygenases involved in the oxidative metabolism of a large variety of compounds as diverse as polycyclic hydrocarbons, drugs, pesticides, and steroids. AHH has been studied primarily in the liver, which is the major source of this enzyme activity, but most other tissues including skin have been shown to have detectable levels of activity (Gielen, Goujon & Nebert, 1972). Interest in cutaneous AHH has been sparked by the fact that the skin is one of the largest organs of the body, and also the first to come into contact with many of the potentially carcinogenic hydrocarbons in the environment. It has been suggested that induction of epidermal AHH in certain experimental situations may have a role in delaying carcinogenesis by polycyclic hydrocarbons (DiGiovanni *et al.*, 1979, 1980).

AHH activity is highly inducible. Among the most effective inducers known is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Poland & Glover, 1974). Poland & Glover (1974) observed that in mouse liver tissue TCDD is 30,000 times more potent than methylcholanthrene in inducing AHH levels and sustaining high levels for up to 35 days.

TCDD is one of the most toxic compounds known. In the guinea-pig, the species most sensitive to its lethal effects, the LD_{50} is around 2 μg/kg body weight (Schwetz *et al.*, 1973). The systemic toxicity of TCDD and its congeners in experimental animals has been correlated with their ability to induce AHH activity (Poland & Glover, 1980; Poland, Glover & Kende, 1976). In humans, an initial effect of TCDD exposure is the development of chloracne (Baughman, 1979). This recalcitrant acneform eruption develops in subjects who have inadvertently been exposed to certain industrial polyhalogenated aromatic hydrocarbons of which TCDD is the prototype. We have been investigating the biochemical changes in hairless mouse skin during experimental induction of chloracne with TCDD (Puhvel *et al.*, 1982). In this study, we report on TCDD-induced changes in cutaneous AHH activity in hairless mice.

METHODS

Animals. Female hairless mice (strain HRS/J), 8 to 10 weeks old at the start of the experiment were obtained from Jackson Laboratories (Bar Harbor, Maine). Animals were housed three to a cage in plastic cages with hardwood bedding and fed laboratory chow *ad libitum*.

Chemicals. Solvents and chemicals were spectrophotometric grade and were used without further purification. TCDD was obtained from KOR, Inc. (Cambridge, Massachusetts). Benzo(a)pyrene was obtained from Sigma Chemicals (St Louis, Missouri). The 3-hydroxybenzo(a)pyrene was a gift from the Chemical Respository (National Cancer Institute, Bethesda, Maryland).

Treatment. TCDD (1 mg/ml of anisole) was diluted in acetone. Animals were treated by topical application of 0·1 μg of TCDD in 0·1 ml of acetone, three times a week for up to 4 weeks. Age-matched controls were treated in the same way with 0·1 ml of acetone alone. At weekly intervals three TCDD-treated animals and three controls were sacrificed by cervical dislocation. After each carcass was carefully rinsed in acetone, a 4-mm punch biopsy of midscapular skin was obtained for histology, and a uniform area (2·5 cm × 3 cm) of dorsal skin removed. The epidermis was separated from the dermis (at 4°C) following heat–cold treatment of whole skin (Thompson & Slaga, 1976) and homogenized in 1 ml ice-cold 0·25 M sucrose, 0·05 M Tris buffer, pH 7·5, for 30 seconds, with a Polytron homogenizer set at 7. This homogenate was the source of the enzyme for the AHH assays and protein determinations.

AHH assays were performed according to methods described by Kinoshita & Gelboin (1972) and Bowden *et al.* (1974). The assays were done in semi-darkness in a total volume of 1 ml which contained 50 μmoles Tris buffer, pH 7·5; 0·5 mg of NADPH; 3 μmoles of $MgCl_2$; 0·2 ml of epidermal homogenate (containing approximately 0·5 mg of protein); and 1 mg of albumin. One hundred nmoles of benzo(a)pyrene was added in 50 μl of methanol to start the incubation. The reaction mixtures were incubated at 37°C with shaking in air for 30 min. The reaction was stopped by adding 4·25 ml of ice-cold hexane–acetone mix (3·25 ml hexane: 1 ml acetone). The flasks were returned to the incubator and shaken vigorously for 10 min and 3 ml of organic phase extracted for 30 seconds with 3 ml of 1 N NaOH on a Vortex mixer. The concentration of 3-hydroxybenzo(a)pyrene extracted was determined spectrofluorometrically in a Varian SF-330 spectrofluorometer, with excitation at 396 nm and emission at 522 nm. The enzyme units were defined as nmoles of 3-hydroxybenzo(a)pyrene formed in 30 min/mg of protein. The spectrofluorometer was always calibrated with a standard curve of 3-hydroxybenzo(a)pyrene, which contained the same reaction mixture but to which hexane–acetone mix was added before benzo(a)pyrene. Enzyme activity was determined in duplicate and compared with blank controls to which hexane–acetone mix was added before benzo(a)pyrene. The soluble protein contents of the epidermal homogenates were determined by the Bradford protein assay (Bradford, 1976).

RESULTS

Morphological evaluation of epidermal changes. TCDD is a potent chloracnegen. In HRS/J mice, dosages of TCDD used in the present study (i.e. 0·1 μg three times a week) induced visibly hyperkeratotic, hyperproliferative epidermis within 2 weeks of treatment. Further treatment progressively exacerbated this condition. Histologically, progressive epidermal hyperkeratosis and hyperplasia were the most obvious changes from the first until the fourth week of treatment. Other effects were loss of sebaceous glands, hyperkeratinization of dermal cysts, and development of a lymphohistiocytic infiltrate in the dermis.

AHH Activity. Results of the AHH assays are summarized in Table 1. In these studies we have expressed AHH activity in relation to mg of soluble protein in the tissue homogenate. As can be seen from Table 1 the water-soluble protein content of hairless mouse epidermis decreased progressively in correlation with increasing hyperkeratinization of the epidermis following TCDD treatment. Untreated hairless mouse skin and skin treated with acetone alone had low innate AHH activity (see Table 2). Twenty-four hours after two consecutive daily applications of 0·1 μg of TCDD, epidermal AHH activity rose to 5·330 ± 0·870 nmoles/mg of protein/30 min. After 1 week (three treatments), this level had decreased to 4·270 ± 1·050 nmoles/mg of protein/30 min, and after 4 weeks of treatment to 0·578 ± 0·248 nmoles/mg of protein/30 min.

Since repeated applications of TCDD appeared to induce progressively lower levels of AHH activity, additional experiments were set up to establish a possible cause for this. It occurred to us that increasing concentrations of TCDD accumulating on the skin of the animals during continued application of the compound over a 4-week period may have interfered with the AHH

TABLE 1. AHH activity in hairless mouse epidermis following repeated topical applications of TCDD

Amount of TCDD applied	Length of application	mg soluble protein/ ml of cutaneous homogenate	AHH activity[a]
0·2 μg	48 hours	2·5 ± 0·56	5·330 ± 0·870
0·3 μg	7 days	2·1 ± 0·72	4·270 ± 1·050
0·6 μg	14 days	1·6 ± 0·46	1·800 ± 0·175
0·9 μg	21 days	1·4 ± 0·41	1·315 ± 0·770
1·2 μg	28 days	1·3 ± 0·21	0·578 ± 0·248

[a] The mean values of three experiments.

Results expressed as nmoles of 3-hydroxybenzo(a)pyrene formed per mg of soluble epidermal protein per 30 min.

assay. To test for this three animals were treated with 0·1 μg of TCDD on two consecutive days, and the skin homogenized for AHH assay as described above.

Duplicate samples of cutaneous homogenates were assayed for AHH activity by using the same assay system as previously described, but adding 1·0 μg of TCDD in 0·1 ml of acetone directly to one of the assay mixtures just prior to incubation. Results of AHH activity were identical with both mixtures indicating that TCDD added directly to the assay mixture did not interfere with AHH activity.

An alternate explanation for decreased AHH activity following prolonged exposure of skin to TCDD may have been related to the changes in cutaneous differentiation which resulted from TCDD application.

The most striking histological changes in TCDD-treated hairless mouse skin were epidermal hyperkeratosis and hyperplasia. Somewhat similar changes can be induced in hairless mouse skin in response to physical or chemical stimuli such as consecutive tape stripping of skin, or by topical application of 50% oleic acid.

Groups of HRS/J mice (six animals to a group) were treated twice daily by topical application of 0·1 ml of 50% oleic acid (in mineral oil) to dorsal skin for 4 consecutive days. On the fifth and sixth days, three animals were treated with 0·1 μg of TCDD in 0·1 ml acetone topically. The others received two consecutive daily applications of 0·1 ml of acetone alone.

A second group of mice underwent tape stripping of dorsal skin. Two-inch strips of tape were applied to the dorsal skins of ether-anaesthetized mice in consecutive applications until a glistening layer of skin was reached (approximately fifteen times). After a 72-hour rest period, half the animals were treated with TCDD, half with acetone, as described above.

Additional control groups consisted of mice which were treated with either TCDD or acetone as described above, but who had not undergone any prior epidermal stimulation. All animals were sacrificed 24 h after the last TCDD or acetone treatment, and the dorsal skin processed for histology and AHH assay as described above.

Results of these assays are shown in Table 2. Epidermis which histologically appeared to be in a proliferative state following tape stripping of stratum corneum, and proliferative and hyperkeratotic after treatment with 50% oleic acid, demonstrated 66% and 42% reduction in AHH inducibility, respectively. To confirm that the treatment regimens employed had induced

TABLE 2. AHH activity in hairless mouse epidermis following various treatments

Procedure	AHH activity[a]
No treatment or treatment with acetone	0·091 ± 0·026
No treatment followed by challenge with 0·2 μg TCDD	4·800 ± 0·780
Tape stripping of stratum corneum	0·077 ± 0·041
Tape stripping followed by challenge with 0·2 μg TCDD	1·640 ± 0·520
50% oleic acid application	0·076 ± 0·020
50% oleic acid followed by challenge with 0·2 μg TCDD	2·280 ± 1·110

[a] AHH activity expressed as the nmoles of 3-OH-benzo(a)pyrene formed per mg of soluble epidermal protein per 30 min.

epidermal proliferation, thymidine incorporation into epidermal DNA was monitored in animals which had been treated by oleic acid application and tape stripping, as just described. Sixteen mice were treated twice daily by topical application of 0·1 ml of 50% oleic acid. Starting at 3 h after applying the oleic acid, and at 24, 48 and 72 h following the initial application, four animals were injected with 30 μCi of [^{3}H]thymidine, sacrificed 1 hour later by cervical dislocation, and the epidermis was processed for DNA synthesis measurements using the method described by Baird *et al.* (1971). Similar experiments were conducted with sixteen animals who underwent tape stripping of stratum corneum. DNA synthesis studies were carried out 3, 24, 48 and 72 h after the tape stripping. At each time four untreated animals were sacrificed and processed as control values for DNA synthesis. Results of tritiated-thymidine incorporation studies are shown in Fig. 1. At the time the animals received their initial TCDD treatment for AHH induction, DNA synthesis was significantly elevated, indicating that the epidermis was in a proliferative state.

DISCUSSION

TCDD is the most powerful inducer of AHH activity known. In epidermis of hairless HRS/J mice, a 52-fold increase in AHH activity was induced by two consecutive daily applications of 0·1 μg. These values were comparable to values reported for AHH activity in skin of Swiss-Webster mice by Pohl, Philpot & Fouts (1976) 24 h after single topical applications of 0·3 μg of TCDD. However, in our experiments continued exposure of skin to TCDD resulted in progressively decreased AHH activity over a 4-week period.

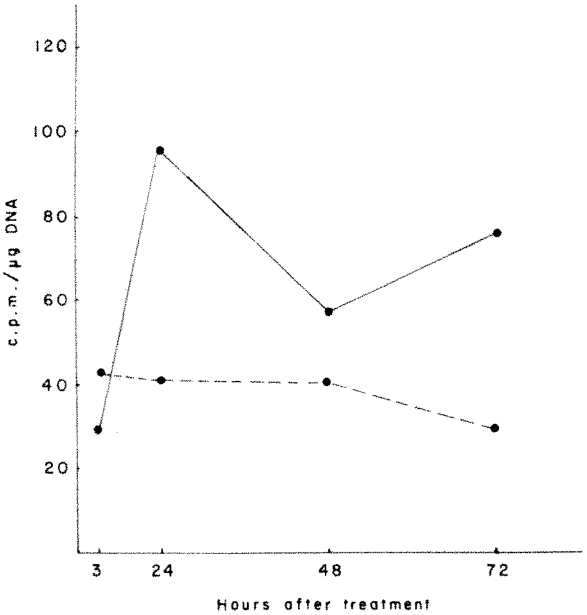

FIGURE 1. Incorporation of [^{3}H]thymidine into cutaneous DNA in control animals (●- - -●) and in mice treated topically with oleic acid, or by tape stripping skin (●——●), expressed as c.p.m. per μg of DNA. TCDD treatment of animals was started at 72 h, at a time when, according to [^{3}H]thymidine counts, the skin was in a hyperproliferative phase.

A possible explanation for this change in cutaneous AHH response seemed to relate to changes in cutaneous differentiation which resulted from prolonged TCDD exposure.

In hairless mice TCDD exposure induced epidermal hyperkeratinization and hyperplasia, squamous metaplasia of sebaceous glands, keratinization of dermal cysts and appearance of an inflammatory infiltrate in the dermis. Changes in AHH activity in such skin could theoretically be related to any of the several morphological changes induced. However, the present studies have dealt only with epidermis. We used heat–cold treatment of skin as described by Thompson & Slaga (1976) to facilitate dermal-epidermal separation. These authors have shown that 30 seconds at 52°C has a negligible effect on mouse epidermal AHH activity. The sebaceous glands remain in the dermis in these preparations. Thus, despite previous findings that the prime site for AHH activity in skin is in sebaceous glands (Wiebel *et al.*, 1975) our findings relate only to epidermal changes.

The relationship between epidermal differentiation and cutaneous AHH activity seemed important in light of the suggestions that cutaneous AHH activity may be significant in 'detoxification' of potentially hazardous hydrocarbons.

Nebert *et al.* (1972) have reported that repeated topical application of 12-*o*-tetradecanoyl phorbol-13-acetate (TPA) depressed basal AHH activity of B_6 or D_6 mice by 30 to 70%, but these authors did not relate this effect to changes in cutaneous morphology. Since topical TPA application induces epidermal proliferation of hairless mouse skin within 48 h (Argyris, 1980), it is possible that the reduction of AHH activity described may have been related to proliferative changes in the epidermis. Similarly, Raumio & Pelkonen (1982) have also noted a decrease in basal AHH activity in rat epidermis during wound healing, a process associated with epidermal proliferation.

These observations together with our own present findings suggest that the state of epidermal differentiation and proliferation may have a direct bearing on AHH response.

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T-cell subpopulations in the blood and skin of patients with psoriasis

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SUMMARY

Monoclonal antibodies were used to determine, simultaneously, the proportions of T-cell populations in the peripheral blood and in the skin lesions of fifty-one patients with psoriasis. The results were analysed in relation to the extent, age and clinical type of the skin lesions. In the group of patients with extensive lesions, a significant reduction in the number of total T (T_T) and T helper/inducer-cells, (T_H), but not in T suppressor/cytotoxic cells (T_S) was observed in the peripheral blood. Furthermore, the skin T_H/T_S ratio was greater in late guttate and in chronic plaque lesions than the corresponding ratio in the blood. These findings suggest that there is an active selective recruitment of T_H cells into established psoriatic lesions. In contrast, the T_H/T_S ratio in early guttate lesions was the same as in the blood, and significantly lower than in the plaque lesions. An additional finding was a decrease of T_S, and a corresponding increase of null cells in the blood of patients with chronic plaque psoriasis. These observations provide further evidence for the participation of T cells in the pathogenesis of psoriasis.

Various studies have attempted to establish an immunological basis for the pathogenesis of psoriasis. Patients with active disease have impaired delayed hypersensitivity (Obalek, Haftek & Glinski, 1977) which corrects with effective therapy (Glinski, Obalek & Haftek, 1978a). A decrease in T-cell numbers has also been demonstrated by several workers (Guilhou *et al.*, 1976; Glinski *et al.*, 1977; Guilhou, Clot & Meynadier, 1977; Glinski *et al.*, 1978b) whilst others have reported normal T-cell blood counts (Levantine & Brostoff, 1975; Lischka, 1976; De Pietro *et al.*, 1981). A decreased suppressor cell function has been described (Hunyadi *et al.*, 1980; Saunder *et al.*, 1980; Gladman *et al.*, 1981), although other studies failed to show a major defect of either suppressor cell subsets or function (DePietro *et al.*, 1981; Clot, Guilhou & Andary, 1982). There is a general agreement that circulating B-cell numbers are normal in psoriasis (Guilhou *et*

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al., 1976; Glinski *et al.*, 1977, 1978b) but an increase in null cells, due to blocking of receptors for sheep erythrocytes by a factor present in serum has been reported in patients with active disease (Glinski *et al.*, 1978b).

Ragaz & Ackerman (1979) have studied the sequence of histological changes in psoriasis. Dilation and tortuosity of the blood vessels in the papillary dermis followed by the appearance of mononuclear cells are the earliest events in the sequence. The dermal mononuclear infiltrate of both early and fully developed psoriatic lesions has been shown to consist almost exclusively of T lymphocytes and macrophages, with very few B lymphocytes or neutrophils (Stingl *et al.*, 1977; Bjerke, Krogh & Matre, 1978).

The recent availability of monoclonal antibodies has allowed the study of T-cell subpopulations in both blood and tissues. The monoclonal antibodies OKT4 and Leu 3a identify T-cell subsets with inducer and helper activities, while the OKT8 and Leu 2a antibodies react with T lymphocytes with cytotoxic and suppressor function. However, the functional diversity of these subsets has probably not yet been fully elucidated.

The aim of the work presented here was to determine whether a consistent relationship existed between these T-cell populations in the blood and skin of psoriasis patients, and whether this correlated with the age, type and extent of the skin lesions.

METHODS

Patients

Fifty-one patients, twenty-four who had chronic plaque psoriasis, and twenty-seven who had guttate psoriasis were studied. Chronic plaque psoriasis was characterized by a slow onset of stable lesions of at least 2 cm in diameter, which had been present for at least 1 year without apparently increasing in size. Guttate psoriasis however, presented as a sudden outbreak of lesions < 5 mm in diameter in the 8 weeks prior to examination, with expansion of existing lesions. Extent of skin lesions was assessed as being < 10% or > 10% of body surface area. Seventeen guttate and twenty chronic plaque psoriatic patients made up the less extensively affected group, while there were ten guttate and four chronic plaque psoriatic patients in the > 10% affected group. There were twenty-nine males and twenty-two females with a mean age of 45 years. All patients had stopped treatment at least 2 weeks before. Controls consisted of twenty-eight healthy adults (hospital personnel): fourteen males, fourteen females.

Peripheral blood

Isolation of lymphocytes. Peripheral blood mononuclear cells (PBM) were isolated by Ficoll-Hypaque gradient centrifugation (Lymphoprep, Nyegaard and Co., A/S, Oslo, Norway). The cells were then washed twice with RPMI 1640 (Flow Laboratories, Irvine, Ayrshire, Scotland) supplemented with 15 mM Hepes, crystamycin (160 µg/ml) and 5% heat-inactivated fetal calf serum. This supplemented medium was used in all subsequent procedures.

Monoclonal antibodies. Two sets of monoclonal antibodies were employed in this study. Firstly, OKT3 (pan T cell), OKT4 (helper/inducer T cell) antibodies (Ortho Pharmaceuticals Corporation, Raritan, N.J.). Secondly, Leu 1 (pan T cell), Leu 2a (suppressor/cytotoxic T cell) and Leu 3a (helper/inducer T cell) antibodies (Becton Dickinson, Sunnyvale, California). In agreement with the findings of others (Reinherz & Schlossman, 1981) each of these antibodies was found to react with the same percentage of T lymphocytes as its OKT counterpart. These two sets of antibodies were used interchangeably in this study and although each subset is functionally heterogeneous they will, for convenience, be designated as T_T (OKT3$^+$, Leu 1$^+$), T_H (OKT4$^+$, Leu 3a$^+$) and T_S (OKT8$^+$, Leu 2a$^+$). Both reagents were used because the Becton Dickinson antibodies were found to be more satisfactory for staining frozen skin sections, whereas the Ortho reagents gave better staining with PBM preparations in our hands.

T-cell enumeration. Five microlitres of a 1:5 dilution of monoclonal antibody were added to 50 μl of a suspension of 5×10^6 lymphocytes/ml, mixed, incubated at 4°C for 30 min and then washed twice with supplemented medium. The cells were then reacted with 25 μl of a 1:10 dilution of fluorescein-conjugated goat anti-mouse IgG (Nordic Immunological Labs., Tilburg, The Netherlands) at 4°C for 30 min. After three washes, the cells were examined under a Leitz (Wetzlar, Germany) fluorescence microscope fitted with an epifluorescence condenser and selective filters for fluorescein isothiocyanate (FITC; green) and tetra ethyl-rhodamine isothiocyanate (TRITC; red).

B-cell enumeration. B cells were counted by direct fluorescent staining of surface immunoglobulin. A pellet of 5×10^6 lymphocytes/ml was resuspended in 25 μl of a 1:10 dilution of rhodamine-conjugated anti-human immunoglobulin and incubated at 4°C for 30 min. After three washes the cells were examined in the fluorescent microscope.

Skin

Skin biopsies. On the same occasion that blood was collected, 2-mm skin punch biopsies were taken from both affected and uninvolved skin. The skin was immediately frozen in liquid nitrogen (embedded in Tissue-Tek II O.C.T. compound, Lamb, London) and stored at −80°C. Five-micron sections were cut on a cryostat (Bright Instrument Co. Ltd., Huntingdon, England), air-dried for at least 30 min, and either stained directly or stored at −80°C.

Lesions from patients with guttate psoriasis were classified as either early (EG) or late (LG) guttate lesions. EG lesions were defined as 2 mm or less in diameter and less than 1 week old, and LG lesions as < 1 cm in diameter and from 1 to 8 weeks old. Biopsies from chronic plaque (CP) lesions were taken from the edge of lesions which were at least 2 cm in diameter and 1 year old.

Detection of T cells

Biotin–avidin–peroxidase method. Skin sections were stained for T cells according to the biotin–avidin–peroxidase method of Warnke, as supplied by Becton Dickinson, with slight modifications.

After air drying, the sections were fixed in acetone at 4°C for 10 min, dried and washed in phosphate-buffered saline (PBS; pH 7·2) for 3 min. The sections were incubated with 5 μl of a 1:5 dilution of monoclonal antibody in a humid atmosphere at room temperature (30 min) washed in PBS (3 min) and then allowed to react for 30 min with 5 μl of a 1:50 dilution of biotin-conjugated goat anti-mouse (Tago, Burlingame, California). After a 3-min wash in PBS and incubation with 5 μl of a 1:10 dilution of avidin–peroxidase (Vector Labs., Burlingame, California) (30 min), the sections were washed twice in PBS (3 min each). They were then developed with diaminobenzidine solution (1–3 mg/ml 3,3-diaminobenzidine (DAB, Sigma) in 0·01% H_2O_2 in PBS) for 8 min, washed twice with PBS (3 min each) and rinsed by dipping ten times in distilled water. A 5-min incubation with copper sulphate solution (0·5% in normal saline) was followed by a rinse in PBS and repeated dipping in distilled water. The sections were counterstained with 1% methylene blue (5 min), dehydrated in 100% ethanol and xylene, mounted in styrolite (Lamb, London) and examined under a light microscope.

The proportions of T_T, T_H and T_S cells present in the dermis of a skin biopsy could, therefore, be determined by examining serial sections stained with Leu 1, Leu 3a and Leu 2a respectively.

Control sections were incubated with avidin–peroxidase with and without biotin-conjugated goat anti-mouse antibody.

B cells. B cells were evaluated by direct immunofluorescence staining of surface immunoglobulin. After air drying the sections were fixed in a chloroform/acetone mixture (1 : 1), washed in PBS (3 min) and incubated with 5 μl of a 1 : 30 dilution of rhodamine-conjugated anti-human immunoglobulin. After two washes in PBS (3 min each) the sections were mounted in 10% PBS in glycerol under a coverslip sealed with nail varnish, and examined under the fluorescence microscope.

Tuberculin skin reaction

Ten healthy volunteers, previously BCG vaccinated, were injected intradermally in the forearm with 0·1 ml of tuberculin PPD (100 units per ml; Evans Medical Ltd., Speke, Liverpool) for the Mantoux test. Blood samples were taken before and 24 hours after injection. Two-mm skin punch biopsies were taken 24 and 48 h after injection from the centre of the lesion.

Expression of results and statistical analysis

Each group of results was expressed as the mean ± standard error. The difference between the means was analysed using the Student's *t*-test.

RESULTS

The findings are summarized in Table 1. Compared with the controls, the psoriatic group as a whole was slightly lymphopenic and this was due entirely to a reduction in T lymphocytes. However, the difference between the two groups was just outside the conventional 5% significance level. Moreover, in the guttate type psoriasis (G), this reduction in T lymphocytes was due entirely to a decrease in T_H. However, in chronic plaque psoriasis (CP) both T_S and T_H were decreased and the T_S reduction was significant at the 5% level. In addition, null cells were significantly increased in these patients.

When psoriatic patients were subdivided according to the extent of the disease, the more extensively affected group showed a significant decrease in T_H cells, while no such correlation was observed in relation to T_S cells. Null cells were also increased in this group but not to a significant level.

Comparison of T_H/T_S ratios in skin and blood

The dermal infiltrate in psoriatic lesions of both clinical types was made up almost entirely of T lymphocytes, B lymphocytes being rarely observed. It was noted that the sum of Leu 2a$^+$ and Leu 3a$^+$ cells was quite often greater than the number of Leu 1$^+$ cells, suggesting an overlap of T_H and T_S markers and/or a selective blocking of the pan T-cell marker.

The mean T_H/T_S ratio in the skin lesions of fifty-one patients with psoriasis was 2·46 ± 0·17 (Table 2). This was significantly greater than the corresponding mean blood ratio of 2·00 ± 0·13 ($P < 0·05$) (Table 2). When the skin T_H/T_S ratios were analysed with respect to the age of the

TABLE 1. Blood lymphocyte populations in psoriatic patients

Lymphocyte population	Controls $n = 28$	Guttate psoriasis (G) $n = 27$	Chronic plaque psoriasis (CP) $n = 24$	Extent of psoriasis < 10% $n = 37$	Extent of psoriasis > 10% $n = 14$
Total lymphocyte count	2257 ± 99	2055 ± 151	2146 ± 171	2167 ± 122	1915 ± 154
Null	337 ± 49	275 ± 43	‡515 ± 54	360 ± 44	482 ± 77
B	356 ± 31	388 ± 46	340 ± 51	391 ± 43	305 ± 53
T_T	1552 ± 91	1352 ± 106	1307 ± 109	1417 ± 75	†1136 ± 163
% T_H	65 ± 1·8	65 ± 2·1	65 ± 2·7	65 ± 2·0	61 ± 3·2
T_H	1021 ± 72	866 ± 76	866 ± 88	927 ± 65	‡706 ± 113
% T_S	36 ± 0·5	39 ± 2·1	34 ± 2·3	36 ± 1·7	39 ± 3·5
T_S	557 ± 44	541 ± 55	*432 ± 43	489 ± 39	469 ± 83

Results are expressed as absolute lymphocyte counts per mm^3 ± standard error of the mean (S.E.) T_H and T_S cells are also expressed as a mean percentage of T_T cells ± s.e.; *n*, number of individuals studied.

Significance levels compared with control va ues: *$P = 0·05$, †$P = 0·02$, ‡$P < 0·02$.

TABLE 2. Comparison of T_H/T_S ratios in blood and skin

Lesion	T_H/T_S ratio Blood	T_H/T_S ratio Skin
Early guttate (< 1 wk) $n = 16$	1.78 ± 0·12	*1·79 ± 0·33
Late guttate (1–8 wks) $n = 28$		2·44 ± 0·25
Chronic plaque $n = 29$	2·25 ± 0·24	*2·85 ± 0·29
Tuberculin reaction, $n = 10$	1·90 ± 0·14	24 h: 2·85 ± 0·66 48 h: 2·16 ± 0·47

* Early guttate *vs* chronic plaque, $P < 0·05$.

lesion (Table 2), a significantly higher ratio was observed for the CP lesions (at least 1 year old) compared with the EG lesions (< 1 week old), whilst the LG lesions had an intermediate T_H/T_S ratio. The T_H/T_S ratios of both the LG and CP lesions were greater than the corresponding ratios in the blood, although these differences did not reach a statistically significant level. The EG lesions had few T cells and their T_H/T_S ratio did not differ from that in the blood. However, preliminary double-labelling experiments have suggested that, judged by the expression of HLA-DR (Ia) antigen, the early lesions contain a relatively higher proportion of activated T_H cells than the LG and CP lesions.

The tuberculin skin reaction was used in an attempt to obtain a set of control skin ratios in non-psoriatic individuals. The T_h/T_S ratios were determined for blood, and in skin reactions of ten healthy adults, 24 and 48 h after an intradermal injection of PPD. At 24 h the mean T_H/T_S ratio in these skin reactions was markedly increased over that observed in blood. At 48 h the total number of T cells had increased, but the T_H/T_S ratio had decreased towards the blood ratio, indicating that between 24 and 48 h, relatively more T_S than T_H cells were recruited to the reaction site (Table 2).

DISCUSSION

The study has demonstrated a decrease in the numbers of T_T and T_H cells in the blood of patients with psoriasis. However, this decrease was significant only in the group of patients with more extensive psoriasis (> 10% surface area involved). In addition, the T_H/T_S ratios of established psoriatic skin lesions were increased compared with the corresponding ratios in the blood. These findings suggest an active and selective recruitment of T_H cells from the blood into psoriatic lesions. Indeed, a significant decrease in blood T_S cells was observed only in patients with chronic plaque lesions, which were generally less extensive and contained relatively fewer T_S cells than the guttate lesions. Moreover, the decrease in T_S cells in CP psoriasis was accompanied by a significant increase in null cells, suggesting that, in these patients, the OKT8 marker may be lost or blocked on a proportion of the cells. A serum factor which can block receptors for sheep erythrocytes on normal blood lymphocytes has been reported in patients with psoriasis (Glinski *et al.*, 1978b). It is possible that such a factor may block other T-cell receptors. A recent study by DePietro *et al.* (1981) using monoclonal antibodies failed to demonstrate any shift in T-cell subpopulations in the blood of nine patients with psoriasis, eight of whom had CP lesions. The absolute numbers of T_T, T_H and T_S reported for the psoriatic patients were, however, similar to those presented here, but because their absolute T_T and T_S normal control values were markedly lower than those obtained by us, the patients' values fell within the normal range.

Although the reduction in the number of circulating T_H cells may largely be a depletion phenomenon, the possibility has not been excluded that a serum factor, blocking the detection of the T_H determinant, may be involved in some patients. Conversely, recruitment into psoriatic lesions may sometimes contribute to the reduction of T_S-cell numbers in the blood. Thus, although both mechanisms may to some extent operate to reduce the numbers of each T-cell subset in the blood of psoriatic patients, we think that emigration is mainly responsible for the T_H-cell reduction and blocking for the T_S-cell decrease.

The implication of these assumptions is that imbalance of immunoregulatory cells in the skin lesions may be a central feature of psoriasis. Thus it is suggested that accumulation of T_H cells, and their interaction with HLA-DR-positive, antigen-presenting cells, may be the principal mechanism whereby the psoriatic lesion is initiated and maintained. The persistence of the lesions would then be due to a relative deficiency of T_S cells, possibly resulting from the appearance of a serum factor impairing the ability of T_S cells to localize and/or function in the lesions. The low T_H/T_S ratio in early guttate lesions is not inconsistent with this hypothesis because our current studies have shown that they contain a relatively high proportion of activated (HLA-DR$^+$) T_H cells. We are currently studying whether resolution of guttate lesions is preceded by a decrease in activated T_H cells and/or a decrease in the T_H/T_S ratio compared with lesions which persist.

Findings similar to those described in this paper have been reported in multiple sclerosis where disease activity correlated with a decrease in the number and function of T_S cells, which are believed to be impaired by a serum factor (Antel *et al.*, 1982). Moreover, high T_H/T_S ratios have been observed in multiple sclerosis lesions (Traugott & Raine, personal communication). Two groups have also recently reported a markedly increased T_H/T_S ratio in synovial biopsies from patients with rheumatoid arthritis, with the T_H cells intimately associated with HLA-DR-positive, antigen-presenting cells (Duke *et al.*, 1982; Klareskog *et al.*, 1982). The implication of these findings is that diseases whose pathogenesis is largely dependent upon local interaction between T cells and macrophages may persist partly because T_S cells fail to counteract this interaction. The cellular kinetics observed in our PPD control skin reactions may be relevant in this context. Thus, while a relative decrease in T_S cells was observed in the psoriasis lesions as they became older, the opposite was found in the self-limiting inflammatory responses induced by the PPD injections.

It is tempting to speculate that a local immunoregulatory imbalance due to failure of T_S cells to localize and become activated in tissue lesions may represent a pathogenic pathway which is common for a variety of persistent diseases with different aetiology. Psoriasis, being a common disease with easily accessible lesions, is a particularly convenient model for sequential studies which may help to elucidate the cellular and molecular processes of such a pathway.

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Inflammatory characteristics of platelet activating factor (PAF-acether) in human skin

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SUMMARY

The phospholipid PAF-acether is released from a range of inflammatory cell types and, in experimental animals, has properties appropriate to a mediator of inflammation. We have studied the inflammatory characteristics of intradermally injected PAF-acether in human skin. An early weal and flare response was succeeded, in a proportion of subjects, by an area of late-onset erythema at the site of the resolved weal, reminiscent of the dual response to allergen in sensitized individuals. The time-course and dose–response relationship of the early response has been determined and a synergistic interaction between PAF-acether and prostaglandin E_2 established. PAF-acether should therefore be considered as a potential mediator of both acute and persisting inflammation in man.

Platelet activating factor (PAF) was first described as a product of IgE-sensitized rabbit basophils, capable of aggregating platelets (Benveniste, Henson & Cochrane, 1972). PAF is an ether-linked analogue of phosphatidylcholine, (1-*o*-alkyl-2-*o*-acetyl-sn-glyceryl-3-phosphorylcholine) and is now referred to as PAF-acether. PAF-acether formation involves phospholipase A_2 activation but PAF-acether is not a product of the arachidonic acid cascade. Formation of PAF-acether from ether-linked phospholipids involves a two-step synthetic pathway; in the first step, there is formation of lyso-PAF, which has similar physicochemical properties to PAF-acether but is biologically inactive; in the second step, lyso-PAF is acetylated to form PAF-acether, which has biological activity (Vargaftig *et al.*, 1981). Following the chemical characterization of PAF-acether, it is now recognized as a product of various inflammatory cell types, including platelets, neutrophils, eosinophils and macrophages, in addition to the basophil, in response to both allergic and non-allergic stimuli (Vargaftig *et al.*, 1981). Apart from well-documented effects on platelets, PAF-acether has a number of other actions appropriate to a mediator of inflammation. It is chemotactic for neutrophils (Basran *et al.*,

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1982a) and will induce aggregation of neutrophils (Czarnetski & Benveniste, 1981) and monocytes (Yasaka, Boxer & Baehner, 1982) as well as producing superoxide anions in the latter cell type.

Local administration of small (pmol) quantities of PAF-acether in the skin or paw of experimental animals elicits acute increased plasma protein extravasation, which, in guinea-pig and rabbit skin, is succeeded by a cellular infiltration within a few hours (Humphrey, Hanahan & Pinckard, 1982; Basran *et al.*, 1982a). In man, intradermal injection of PAF-acether induces an acute weal and flare response, succeeded by an area of erythema of late onset at the site of the resolved weal (Basran *et al.*, 1983). The ability of a chemically defined mediator to cause biphasic inflammatory responses has not been described hitherto and is of particular interest in the context of allergic inflammation since, in certain sensitized individuals, intracutaneous injection of allergen produces a similar dual inflammatory response (Dorsch & Baur, 1980).

In the present study, we have defined the dose–response relationship and time-course of inflammatory responses to PAF-acether in the skin of human volunteers and have established an interaction between PAF-acether and prostaglandins.

METHODS

Ethanolic stock solutions of synthetic PAF-acether (a gift from Professor Godfroid and Dr J.Benveniste, INSERM, U200, Paris), Lyso-PAF (Bachem), and prostaglandin E_2 (PGE_2; Prostin E2, Upjohn Ltd.) were diluted in sterile phosphate-buffered saline (PBS; Gibco) for intradermal injection.

Healthy, non-atopic volunteers (aged 20 to 36 years) gave their informed consent to participate in this study. Cutaneous weal and flare responses were induced by intradermal injection of a fixed volume (50 μl) of PBS containing PAF-acether (30–200 ng/site); injections of PBS either alone or containing lyso-PAF (200 ng/site) provided appropriate controls. Interaction studies were performed by intradermal injection of 50 μl of PBS containing PAF-acether (30 ng/site), PAF-acether (30 ng/site) admixed with PGE_2 (0·5 μg/site), PAF-acether (60 ng/site) or PGE_2 (0·5 or 1 μg/site).

To minimize bias, solutions were coded and assigned randomly to marked sites on the flexor surface of both forearms according to a Latin square design. For intradermal injections 25 gauge needles were used and cutaneous responses were measured as weal volume and weal and flare areas. A low tension, spring-loaded thickness gauge (Mitutoyo, Japan) provided reproducible measurements of skinfold thickness (mm). Measurements were made immediately prior to injection (T_0) and after weal formation (T_1) (usually at 15 minutes, the time of maximal response). Two perpendicular weal diameters (D_1 and D_2; mm) were measured and weal volume was calculated as

$$(\text{area} \times \text{depth}) = \frac{\pi}{4}\left(\frac{D_1 + D_2}{2}\right)^2 \times \frac{(T_1 - T_0)}{2}\ \mu\text{l}.$$

Flare area was calculated from two perpendicular diameters (d_1 and d_2; cm):

$$\text{area} = \frac{\pi}{4}\left(\frac{d_1 + d_2}{2}\right)^2 \text{cm}^2$$

usually measured at 5 minutes, the time of maximal response. The onset and duration of late-onset erythema at the site of the resolved weal were recorded.

RESULTS

Effect of PAF-acether

Intradermal injection of PAF-acether (30 and 100 ng/site) induced weal responses that attained maximal volume within 15 minutes; significant responses were still evident at 30 minutes. In six of eight subjects, weal responses to PAF-acether had disappeared within 1 hour and, in the remaining subjects, within 90 minutes (Fig. 1). The area of the flare response was maximal 5

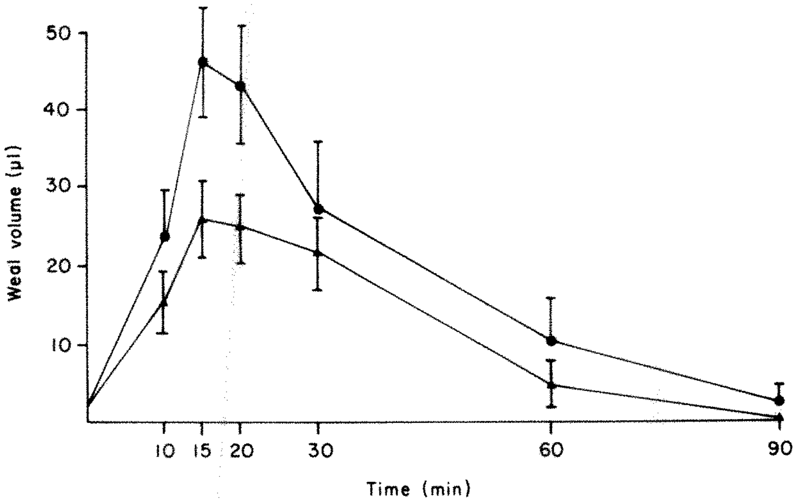

FIGURE 1. The time course of the weal volume response to intradermal PAF-acether, 30 ng (▲) or 100 (●) in 50 μl saline. Each point shows the mean weal volume response and vertical bars represent the standard errors (n = 8).

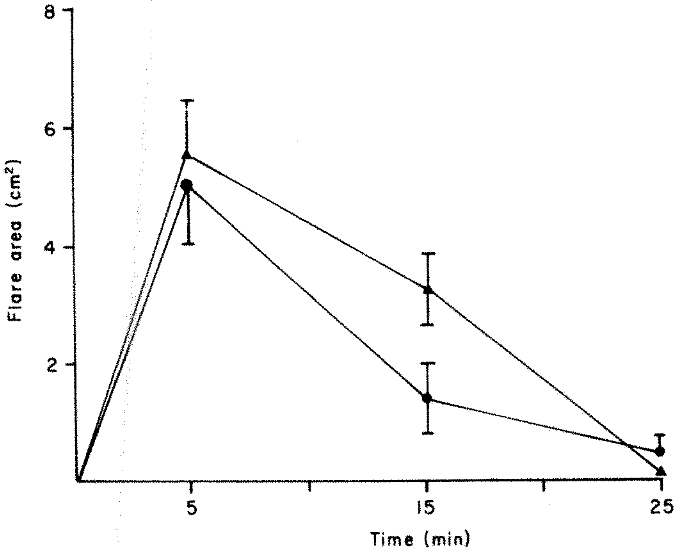

FIGURE 2. The time course of the flare area response to intradermal PAF-acether, 30 ng (▲) or 100 ng (●) in 50 μl saline. Each point shows the mean flare area response and vertical bars represent the standard errors (n = 8).

minutes after administration, and resolved within 25 minutes (Fig. 2). In all subjects, PAF-acether (30–200 ng/site) elicited a dose-related, acute weal response expressed as weal volume (Fig. 3). The flare area response over this dose range was not so clearly dose-related.

Following administration of PAF-acether (100 and 200 ng/site), the early response was succeeded, after an interval of 3–6 hours, by an area of erythema at the site of the resolved weal, in 54% (13/24) of subjects tested. In four subjects who exhibited a late-onset response, this phenomenon was observed on each occasion of repeated administration (at intervals of at least 2 weeks). This late onset response was sometimes accompanied by hyperalgesia at the site of erythema. Hyperalgesia and erythema usually resolved within 1–2 hours of appearance.

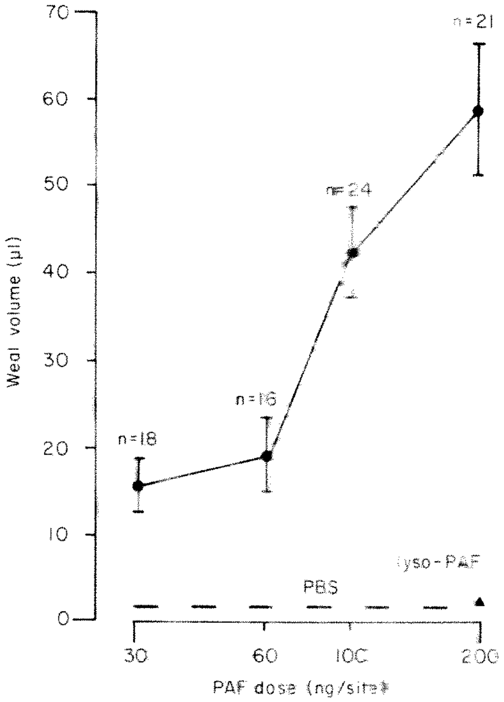

FIGURE 3. The dose–response relationship for PAF-acether on weal volume. Weal volumes were measured 15 min after intradermal injection of PAF-acether (●; 30–200 ng in 50 µl saline; n = 16 to 24). Responses produced by intradermal injection of saline (- - -; PBS; n = 24) or lyso-PAF (▲; 200 ng; n = 10) are also shown. Each point shows the mean weal volume response and vertical bars represent the standard errors (n = 10 to 24) where these exceed the size of the symbols.

Effects of lyso-PAF

Intradermal administration of the phospholipid, lyso-PAF (200 ng/site) produced no acute response in any subject tested (n = 10) in comparison with PBS alone (Fig. 3). No delayed onset response was observed following injection of lyso-PAF (n = 10).

Effect of PGE_2

PGE_2 (0·5 µg/site) produced a modest weal volume response and a prominent flare response (Fig. 4). Both weal volume (70 ± 9 µl; n = 10) and flare area (16 ± 2 cm^2) responses to the mixture of 30 ng PAF-acether and 0·5 µg PGE_2 were significantly greater (weal volume, $P < 0{\cdot}001$; flare area, $P < 0{\cdot}05$; paired t-test) than could be accounted for by mere summation of the responses to

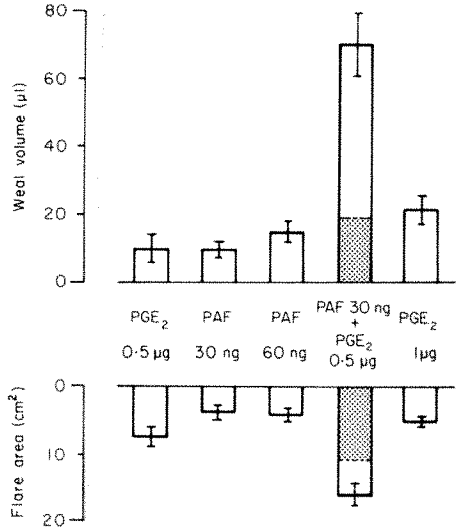

FIGURE 4. Interaction between prostaglandin E₂ (PGE₂) and PAF-acether (PAF). Flare areas (cm²) and weal volumes (µl) were measured 5 and 15 min, respectively, after intradermal injection of PGE₂ (0·5 or 1 µg), PAF (30 or 60 ng) or PAF + PGE₂ (30 ng + 0·5 µg). Each histogram block indicates the mean response and vertical bars represent the standard error (n = 10). The shaded area represents the mean response obtained by summing the individual responses to PGE₂ (0·5 µg) and PAF (30 ng).

the individual components (weal volume, 19 ± 4 µl; flare area, 11 ± 2 cm²). The responses to the mixture of PAF-acether and PGE₂ were also significantly greater ($P < 0{\cdot}001$ in all cases) than responses to a twofold increase in the concentration of either PAF-acether (60 ng/site; weal volume, 14 ± 3 µl; flare area, 4 ± 1 cm²) or PGE₂ (1 µg/site; weal volume, 21 ± 4 µl; flare area, 5 ± 1 cm²) (Fig. 4).

DISCUSSION

Preliminary studies in human volunteers have indicated that intradermal injection of PAF-acether elicits an inflammatory response with both acute (Pinckard *et al.*, 1980; Basran *et al.*, 1983) and late-onset (Basran *et al.*, 1983) components. This double-blind investigation has confirmed and extended these observations.

PAF-acether produces dose-related weal and flare responses in human skin, whereas lyso-PAF produces no discernible response. It seems likely, therefore, that the effects of PAF-acether reported here are as specific as those observed with this molecule in other biological systems. As PAF-acether is known to cause platelet-dependent biological effects (Vargaftig *et al.*, 1981), participation of platelets in the acute inflammatory response has not been excluded. However, it is known that vessel wall permeability changes in the skin of experimental animals are independent of platelet aggregation (Butler *et al.*, 1983; Pirotzky *et al.*, 1983). It is now well established that there are synergistic interactions between permeability factors and prostaglandins in acute inflammation, both in experimental animals (Williams & Morley, 1973; Williams, 1979; Morley, Page & Paul, 1981) and man (Basran *et al.*, 1982b). The acute inflammatory response to PAF-acether in man is potentiated by PGE₂, in agreement with the synergistic interaction between PAF-acether and prostaglandins in guinea-pig and rabbit skin (Wedmore & Williams, 1981; Dewar *et al.*, 1983).

In a proportion of subjects, PAF-acether (more than 100 ng/site) elicits a biphasic response. This is an unusual phenomenon that has not been described as a property of other proposed mediators of allergy and inflammation. The biphasic response is of considerable interest in the context of allergic inflammation, for the time-course of PAF-acether-induced inflammatory responses is reminiscent of the dual response observed to allergen in sensitized individuals (Dorsch & Baur, 1980). Furthermore, intradermal injection of PAF-acether in guinea-pigs induces, by 24 hours, a substantial infiltrate of inflammatory cells comprising predominantly neutrophils, but also including mononuclear cells (Basran *et al.*, 1982a). Preliminary studies in human skin have demonstrated similar inflammatory cellular infiltrates, with a perivascular infiltrate from 4 hours onwards comprising lymphocytes and histiocytes in addition to neutrophils (Archer *et al.*, 1983). In the light of these observations, PAF-acether should be considered as a potential mediator of both acute and persisting inflammation in skin and other tissues.

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Ultrastructural study of iron deposition in the eccrine sweat glands of patients with iron overload

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SUMMARY

An ultrastructural study of eccrine sweat glands was carried out on three patients with iron overload (two with idiopathic haemochromatosis and one with transfusional haemosiderosis). In all three patients small electron-dense particles were seen in both eccrine sweat glands and macrophages. They appeared primarily in the clear cells of the eccrine sweat glands as membrane-bound irregularly shaped bodies, and were shown to contain iron by energy dispersive X-ray microanalysis. Iron aggregates in and around sweat glands were also seen on light microscopy.

Iron deposition in the skin of patients with idiopathic haemochromatosis (IH) is characteristically present in the cells of eccrine sweat glands. It is said to be specific for this condition, and it does not occur in normal subjects (Chevrant-Breton *et al.*, 1977). Electron microscopy of these iron deposits has not been reported. We have performed an ultrastructural study on the sweat glands of two patients with IH and one with sickle cell anaemia who was overloaded with iron from repeated transfusions.

METHODS

Two patients with IH were selected. Both had clinical and laboratory markers of iron overload and a positive family history for the disorder. Both had liver and bone marrow biopsies showing iron accumulation in parenchymal cells. Patient 1 was untreated and newly diagnosed, while patient 2 had repeated therapeutic phlebotomies for 12 years. Patient 3 was a woman with sickle cell disease and laboratory and clinical evidence of iron overload after multiple blood transfusions over many years. Her liver biopsy also showed massive accumulation of iron in both parenchymal and Kupffer cells. Table 1 summarizes the findings in these three patients.

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Light microscopy

Four-millimetre punches of forearm skin were embedded in paraffin wax and stained with Prussian blue.

Electron microscopy

Four-millimetre punches of forearm skin were minced and fixed by immersion in 2% paraformaldehyde with 2·5% glutaraldehyde in 0·1 M cacodylate–HCl, buffered at pH 7·4 (Karnovsky's solution) at room temperature for 2 h, post-fixed in 2% osmium tetraoxide for 1 h, dehydrated in a graded series of cold ethanol baths, and then embedded in Epon 812. One-micron sections were cut for locating the eccrine sweat gland. Thin (500-Å) sections were cut with a diamond knife on a Porter-Blum MT-2 microtome, and stained with a 7% saturated alcoholic solution of uranyl acetate for 30 min in the dark, and counter-stained in a 2% solution of bismuth subnitrate diluted 1 : 50 for 30 min. The sections were then examined in a Hitachi 12A electron microscope.

Energy dispersive X-ray microanalysis

Thin sections were observed in a JEOL 100CX TEMSCAN, operating at 80 kV. X-ray spectra were collected for 60 seconds with a stationary 500-Å diameter beam at a 45° tilt angle using a Princeton Gamma Tech (PGT) detector (30 mm²). Qualitative analysis of the spectra was performed using a PGT System III analyser and software. X-ray spectra from adjacent areas of the embedding media served as controls.

RESULTS

Light microscopy

Aggregates of haemosiderin deposits were seen in and around eccrine sweat glands in all three patients. Macrophages also contained some haemosiderin deposits.

Transmission electron microscopy

The eccrine sweat glands appeared normal. Clear (serous) and dark (mucous) cells could be easily identified. Macrophages and other cells were found in the area adjacent to the sweat glands. Electron-dense particles were seen in both sweat glands and macrophages. Most of the particles in the eccrine sweat glands were round to ovoid, and were found in clusters (Fig. 1). They were seen primarily within the clear cells. Numerous membrane-bound bodies filled with a fine, particulate, electron dense material were noted in the macrophages (Fig. 2). These bodies

TABLE 1.

	Patient 1	Patient 2	Patient 3
Age	58	62	32
Sex	M	M	F
Diagnosis	IH	IH	Sickle cell anaemia
Serum iron	285 µg/dl	142 µg/dl	292 µg/dl
% Transferrin saturation	92	73	96
Serum ferritin	2500 ng/ml	44 ng/ml	10,000 ng/ml

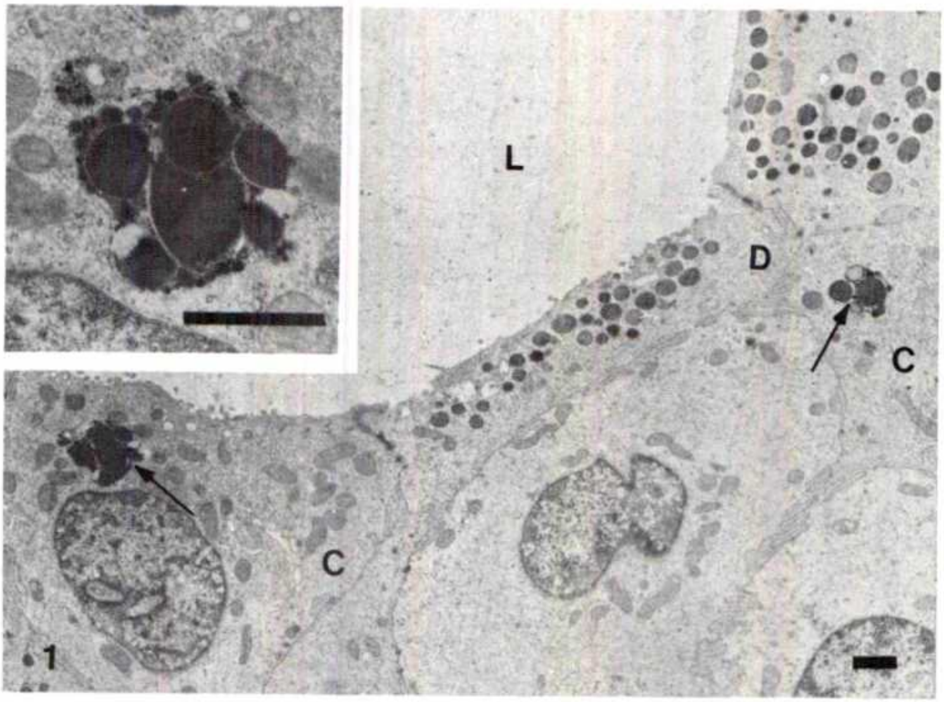

FIGURE 1. Eccrine sweat gland showing electron-dense particles (arrows) in the clear cells (C). D = dark cell, L = luminal. (× 5300.) Bar = 1 μm. Inset: high magnification of the particles shown by the left arrow. (× 18,400.) Bar = 1 μm.

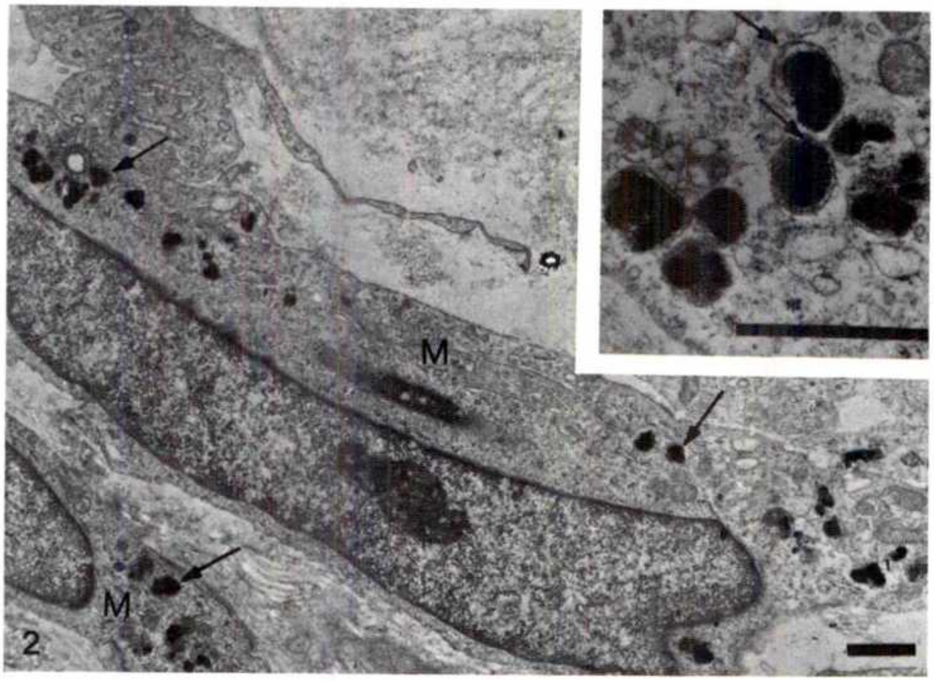

FIGURE 2. Two macrophages (M) with electron-dense particles (arrows) in them. (× 9000.) Bar = 1μm. Inset: high magnification of these particles showing membranes (arrows) around them. (× 26,000.) Bar = 1 μm.

were generally smaller than those seen in sweat glands, and their content was more condensed. Generally, several clusters of electron dense material could be seen within one membrane-bound body.

In the skin of the sickle cell anaemia patient iron deposits were found both in the sweat glands and macrophages. They appeared the same as those seen in the haemochromatosis patients.

Energy dispersive X-ray microanalysis

Electron-dense particles in the sweat glands and macrophages contained iron as determined by energy dispersive X-ray microanalysis, while in the control areas (regions containing no electron-dense material) no iron peak was detected (Figs 3 and 4). Thus, these particles should be considered as siderosomes.

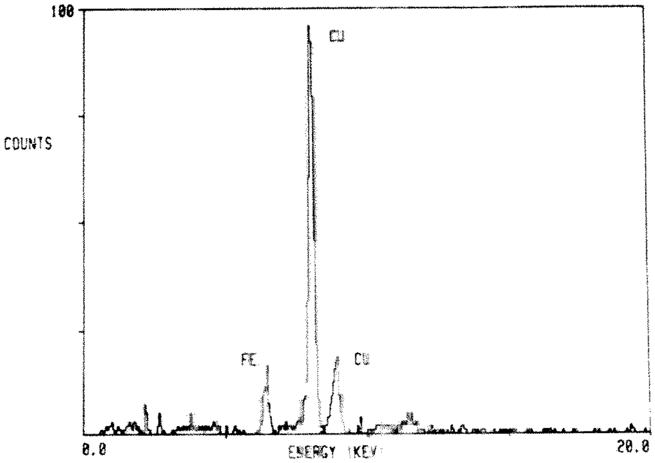

FIGURE 3. X-ray spectrograph taken from microarea over an electron-dense particle in the clear cell of an eccrine sweat gland shows a prominent iron peak (FE). CU = peaks from copper grid.

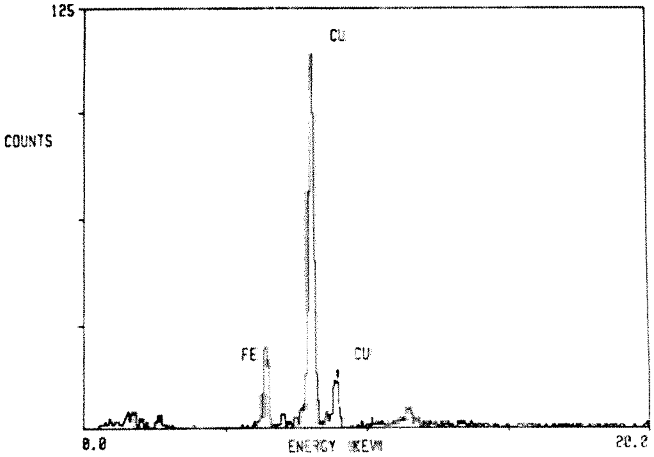

FIGURE 4. X-ray spectrograph taken from microarea over an electron-dense particle in a macrophage shows a prominent iron peak (FE). CU = peaks from copper grid.

DISCUSSION

Idiopathic haemochromatosis is a disease characterized by progressive and massive deposition of iron in tissues (Finch & Finch, 1955). Though familial, not all affected relatives need have the same degree of organ involvement and derangement (Feller *et al.*, 1977). The accumulation of iron in specific organs, such as the skin, liver, pancreas, and heart leads to the classical clinical presentation of patients with bronze hyperpigmentation, cirrhosis, diabetes mellitus, and heart failure (Grace & Powell, 1974).

It is known that the skin hyperpigmentation is generally due to melanin deposition rather than iron alone (Robert, Zurcher & Schmidli, 1950), though the latter has been postulated to be the precipitating event (Robert, Zurcher & Schmidli, 1953). Although much has been written on the validity of non-invasive tests in making the diagnosis and in predicting affected relatives (Wands *et al.*, 1976), the usefulness of serum iron, transferrin saturation, serum ferritin, HLA typing, and chelatable iron are still very much debated when compared with the actual demonstration of iron in the parenchymal tissue of the liver. The latter remains the definitive test for this puzzling disorder (Powell & Halliday, 1978).

In order to avoid the relative risk of a liver biopsy, it has also been thought that a skin biopsy may be useful and specific for this disease (Chevrant-Breton *et al.*, 1977). Skin biopsies of many, but not all, patients with IH show the iron to be deposited in and around the eccrine sweat glands. It does not occur in normal subjects (Weill & Weill-Busson, 1966). Similar studies have not been done on patients with secondary haemosiderosis. This condition usually results from accumulation of iron after repeated blood transfusions for the treatment of chronic anaemias. Though it closely resembles IH in its clinical and laboratory findings, it differs from IH in that the deposition of iron is in the reticuloendothelial system first, before the eventual demonstration of iron in the parenchymal cells (Baldus *et al.*, 1978). Therefore, the sequence of iron deposition appears to be the reverse of that which occurs with IH.

Our three patients all had clinical and laboratory evidence of iron overload and in all three the iron was aggregated in membrane-bound irregularly shaped bodies. The clear cells, perhaps because of their role in handling electrolytes (Sato & Dobson, 1970), were more heavily involved. We also found similar deposits in macrophages, as described in macrophages in other tissues (Yam *et al.*, 1968). It is of interest that our patients with transfusional haemosiderosis had the same light and electron microscope features of iron deposition as in IH. This suggests that the involvement of sweat glands is not specific for IH as previously reported (Weill & Weill-Busson, 1966) but may be a feature of any severe iron overload state.

The pathophysiology of the formation of these iron deposits is unknown; it is possible that they represent an attempt by the body to remove the excess iron. In the salivary glands of both IH (Blandford *et al.*, 1979) and secondary haemosiderosis (Ward-Booth, Ferguson & MacDonald, 1981) similar iron deposits have been shown to interfere with the proper function of the affected glands. Quantitative studies of iron excretion would be helpful to analyse this problem in eccrine sweat glands.

We think that the particles of iron deposition that we have demonstrated are a helpful index of iron overload states, and may provide diagnostic help in patients affected by these disorders.

ACKNOWLEDGMENTS

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X-ray microanalysis. The energy dispersive X-ray microanalysis in this work was supported by NIH grants AM 28143 and AM 19525.

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HLA antigen frequency in granuloma annulare

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SUMMARY

The frequency of HLA-A and -B antigens in fifty-nine patients with localized granuloma annulare was compared with the frequency in 200 blood donors. The frequencies of HLA-A29, -B14 and -B15 were increased significantly in the patient group, the increase of HLA-A29 remaining significant after correction for the number of antigens compared.

Previous reports have produced conflicting evidence as to whether there is a relationship between granuloma annulare and diabetes mellitus (Rhodes *et al.*, 1966; Romaine, Rudner & Altman, 1968; Dicken, Carrington & Winkelman, 1969; Mobacken, Gisslén & Johannisson, 1970). A significant increased frequency of HLA-B8 in patients with granuloma annulare (Andersen & Verdich, 1979) prompted the authors to suggest that there may be a relationship between granuloma annulare and insulin-dependent diabetes mellitus (IDDM) which has been shown to have an increased frequency of HLA-B8 and -B15 (Cahill & McDevitt, 1981). The present report describes the tissue typing of fifty-nine patients with localized granuloma annulare.

METHODS

Fifty-nine patients with granuloma annulare were tissue typed for HLA-A and -B locus antigens. The patients included in the study had all been diagnosed in the Belfast area Dermatology Clinics as typical cases of localized granuloma annulare. Where any doubt of the diagnosis existed, the patients were excluded. There were no known cases of diabetes mellitus among the patients studied. Sera defining the following antigens were either supplied by the National Tissue Typing Reference Laboratory, Bristol, or were antisera of our own which had been checked for their specificity in other laboratories.

HLA-A1, A2, A3, A9, A10, A11, A28, A29
HLA-B5, B7, B8, B12, B13, B14, B15, B17, B18, Bw22, B27, Bw35, B40

Tissue typing was by a standard two-stage microlymphocytotoxicity test at 22°C and at least

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three sera were used to define each antigen. The frequencies of the HLA antigens in the patients were compared with those in 200 blood donors from Northern Ireland (Middleton & Martin, 1978). The significance of differences between the two groups was assessed by a Yate's corrected χ^2 test.

RESULTS

The frequency of HLA-A and HLA-B antigens in fifty-nine patients with localized granuloma annulare and in the control group is shown in Table 1. Only χ^2 values greater than four are given. The frequency of the HLA antigens A29, B14 and B15 differed significantly in patients from their respective frequencies in the control population. The difference for A29 remained significant after correction for the number of antigen comparisons made. The combination HLA A29/B12 occurred in thirteen patients and in ten controls. This finding is statistically significant.

TABLE 1. Frequency of HLA antigens in patients with granuloma annulare and in blood donors from Northern Ireland

HLA antigen	Granuloma annulare (n = 59) No.	%	Blood donors (n = 200) No.	%	χ^2	*P*
A1	26	44·1	86	43·0		
A2	28	47·5	91	45·5		
A3	14	23·7	53	26·5		
A9	7	11·9	31	15·5		
A10	4	6·8	20	10·0		
A11	5	8·5	28	14·0		
A28	4	6·8	10	5·0		
A29	15	25·4	16	8·0	11·524	0·0007 (0·0144)
B5	1	1·7	13	6·5		
B7	17	28·8	67	33·5		
B8	18	30·5	69	34·5		
B12	23	39·0	54	27·0		
B13	2	3·4	5	2·5		
B14	2	3·4	30	15·0	4·650	0·0311 (0·6521)
B15	8	13·6	9	4·5	4·709	0·0300 (0·6301)
B17	8	13·6	18	9·0		
B18	6	10·2	16	8·0		
B22	0	0	8	4·0		
B27	2	3·4	11	5·5		
B35	4	6·8	16	8·0		
B40	5	8·5	20	10·0		
A29 with B12	13	22·0	10	5·0	14·300	0·0002 (0·0032)

Only χ^2 values > 4 are recorded. *P* values in parentheses have been corrected for the number of antigen frequencies compared.

DISCUSSION

Reports of antigens of the HLA-A locus being associated with diseases have usually been explained by their linkage disequilibrium with an antigen from the HLA-B locus. In this study HLA-B12 was increased, although not significantly, in the patient group (39%) compared with the control group (27%). HLA-A29 is known to be in linkage disequilibrium with HLA-B12 (Bodmer & Bodmer, 1978), and the increase in the frequency of HLA-A29 in the patients may be due to the increase in the frequency of HLA-B12, as of the fifteen patients with HLA-A29, thirteen had also HLA-B12.

In the study of Andersen & Verdich (1979) the antigen HLA-B8 was present in 39·5% patients with granuloma annulare. This frequency was significantly higher than in the control population (23·7%). This finding could not be confirmed in the present study for although there is a high frequency of HLA-B8 in a Northern Ireland population (Middleton & Martin, 1978) (34·5%) the frequency in the patients with granuloma annulare was 30·5%.

However, one of the antigens (B15) which was increased in the patients with granuloma annulare is also increased in IDDM. Unfortunately this increase was no longer significant after correction for the number of antigens tested. It would be of interest to find out if either or both DR3 and DR4 are increased in patients with granuloma annulare as these two antigens have been reported to be increased in IDDM and are associated with B8 and B15 respectively.

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Difficulties in producing antibodies to purified *Propionibacterium acnes* exocellular enzymes

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SUMMARY

Attempts were made to produce antisera to purified preparations of *Propionibacterium acnes* lipase, hyaluronate lyase and acid phosphatase in rabbits. Antiserum to lipase (neutralizing titre 1:32) was produced using conventional methods. Lipase (30 μg) in Freund's complete adjuvant (FCA) was injected into multiple sites thrice at weekly intervals. Antibody levels were boosted by i.v. injections of 30 μg in saline at 2-weekly intervals for 2 months. Such regimes failed to raise antibodies to hyaluronate lyase and acid phosphatase.

In order to produce antiserum to hyaluronate lyase, rabbit mononuclear cells were incubated with 45 μg of hyaluronate lyase *in vitro* (1 h at 37°C) prior to injection subcutaneously above the rabbit ankle joint. Simultaneous injections of hyaluronate lyase in FCA (45 μg) were administered into the footpad and thigh. Three treatments at weekly intervals produced an antiserum with a neutralizing titre of 1:256. A similar regime failed to raise antibodies to acid phosphatase.

Antibodies to *P. acnes* lipase and hyaluronate lyase also neutralize the enzymes produced by *P. granulosum*. The neutralization curves suggest that the enzymes produced by both species are related antigenically but not identical.

It has been postulated that *P. acnes* and *P. acnes* exocellular products are implicated in the initiation and persistence of inflammatory acne vulgaris (Holland, Ingham & Cunliffe, 1981). *P. acnes* produces a variety of exocellular enzymes *in vitro*, the most extensively studied being the lipase, hyaluronate lyase and acid phosphatase (Holland *et al.*, 1981). The role of these exocellular enzymes in the physiology of *P. acnes in vivo* and in the pathogenesis of inflammatory acne can only be properly assessed by determining whether these activities are present in normal pilosebaceous follicles and acne lesions. Fluorescein-labelled antibodies to *P. acnes* exocellular enzymes are potentially of use to determine whether the enzymes are present in the follicles of

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both normal and acne skin. Therefore, we have attempted to produce antisera to purified preparations of *P. acnes* lipase, hyaluronate lyase and acid phosphatase in rabbits.

MATERIALS AND METHODS

Bacteria

The exocellular enzymes were elaborated by *P. acnes* strain P-37 (Type I of Marples & McGinley, 1974). *P. granulosum* lipase and hyaluronate lyase were produced by strain FC 208 ii.

Purification and assay of exocellular enzymes

Hyaluronate lyase, acid phosphatase and lipase were purified from culture supernatants after growth of P-37 in Brain Heart Infusion (Difco) and assayed according to Ingham *et al.* (1979, 1980, 1981).

Animals

New Zealand White female rabbits (initial weight 2·5–3 kg) were used.

Neutralization of exocellular enzymes

Test blood was drawn by cardiac puncture and the serum obtained. Serum (0·5 ml) was incubated in doubling dilutions in PBS with a standard dose of enzyme in 0·5 ml PBS (0·3 units of lipase; 0·015 units of hyaluronate lyase or acid phosphatase). Enzyme activity was determined before and after 1 h at 37°C by removing 0·4 ml into the standard assay reaction mixtures. Positive (enzyme in PBS) and negative (serum in doubling dilutions in PBS) controls were included. Tests were carried out in duplicate. The neutralizing titre was defined as the highest dilution of antiserum causing a 50% reduction in enzyme activity after 1 h at 37°C.

Immunization procedures

Intravenous (i.v.) inoculation. Enzyme (10, 12 or 30 μg) in saline (1 ml) was injected into the rabbit ear vein.

Intramuscular (i.m.) inoculation in Freund's complete adjuvant (FCA). Enzyme (10 or 12 μg) in saline (1 ml) was emulsified in an equal volume of FCA (Difco; containing *Mycobacterium butyricum*). Doses of 0·5 ml were injected into the hind and fore quarters.

Multiple inoculation in FCA. Enzyme (30 μg) in saline (2·5 ml) was emulsified in an equal volume of FCA. Ten injections of 0·5 ml were administered; six, intramuscularly into each hind footpad, thigh and hind quarter and four subcutaneously on either side of the back at the shoulders and base of spine.

Subcutaneous (SC) inoculation with 'activated' mononuclear cells plus simultaneous i.m. inoculation. Heparinized blood (10 ml) was drawn from the rabbit by cardiac puncture and diluted 1:1 with saline. The mononuclear cell fraction was separated using a sodium metrizoate/Ficoll gradient (Lymphoprep; Nyegaard & Co). The mononuclear cells were resuspended and washed twice in culture medium (RPMI; Gibco). Approximately 10^8 mononuclear cells were then incubated in PBS (1 ml; pH 7·4) for 20 min at 37°C. Enzyme (30 or 45 μg) in PBS (1 ml) was then added and the mixture reincubated for 1 h at 37°C. The cells were then washed and resuspended in PBS (1 ml) and injected in two 0·5-ml doses subcutaneously directly above the ankle joints in each hind limb. Simultaneously, enzyme (30 or 45 μg) in FCA (total volume 2 ml) was injected into each hind footpad and thigh muscle.

RESULTS

P. acnes (strain P-37) produced only 10 μg (acid phosphatase) and up to 30 μg (lipase and hyaluronate lyase) per litre of culture supernatant. Attempts to produce antisera to the enzymes were therefore limited by the quantities of the enzymes available for immunization regimes.

Antibody responses to exocellular enzymes administered by i.v., i.m. and multiple inoculation regimes

Firstly, immunization regimes of i.m. and i.v. inoculations were undertaken. Ten (acid phosphatase and hyaluronate lyase) or 12-μg (lipase) doses were injected i.m. four times at monthly intervals followed by equal doses i.v. at 1- to 2-weekly intervals for 7 months. This regime failed to raise significant levels of neutralizing antibodies to the three enzymes. A slight response to lipase was observed at three months (Fig. 1a).

Secondly, attempts were made to raise antibodies by increasing the dosage of enzymes to 30 μg and injecting the enzyme at multiple sites three times at weekly intervals followed by equal doses i.v. at 1- to 2-weekly intervals. This regime produced significant neutralizing antibodies to lipase (Fig. 1b). Titres of 2^{-6} (1:64) were observed after 2 months, however these titres could not be increased by subsequent i.v. injections. Similar regimes failed to produce neutralizing antibodies to the other enzymes tested.

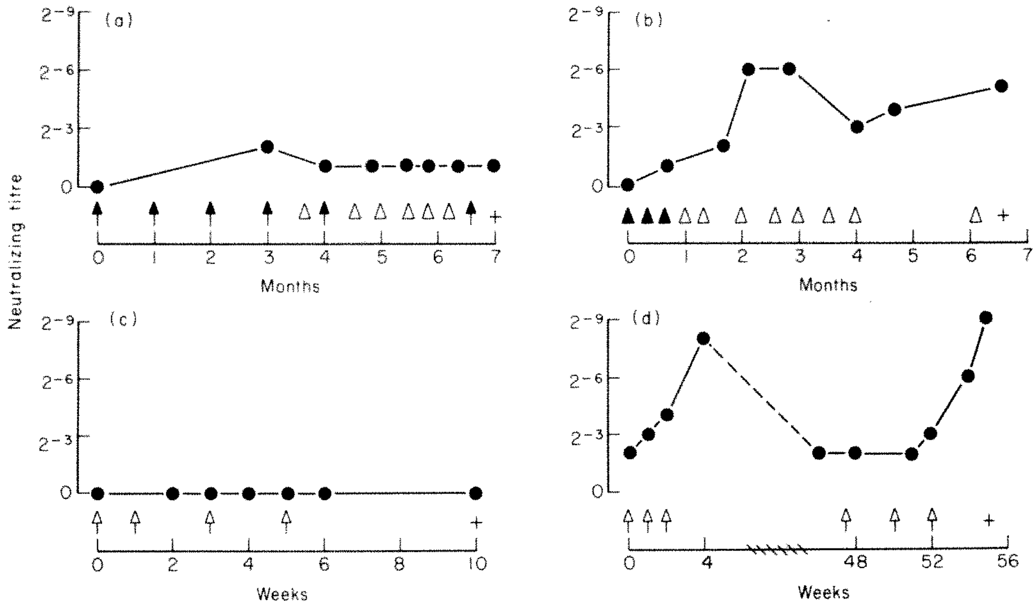

FIGURE 1. Antibody response to *P. acnes* exocellular enzymes in rabbits using various inoculation regimes: (a) lipase 10-μg doses; (b) lipase 30-μg doses; (c) Acid phosphatase 60-μg doses; and (d) hyaluronate lyase 90-μg doses. ↑, i.m. inoculation; △. i.v. inoculation; ▲, multiple inoculation; ⇧, subcutaneous inoculation with 'activated' mononuclear cells and simultaneous inoculation i.m.; and + sacrificed.

Antibody responses to exocellular enzymes after SC inoculation of 'activated' mononuclear cells plus i.m. inoculation

Due to the lack of success using conventional inoculation regimes this novel method was tested in an attempt to produce antisera to acid phosphatase and hyaluronate lyase. The maximum amounts of these enzymes which could be produced in the laboratory were used. Enzyme (30 μg of acid phosphatase; 45 μg of hyaluronate lyase) was incubated with rabbit mononuclear cells *in vitro*. The 'activated' mononuclear cells were injected SC at the ankle joint in an attempt to produce a 'focus' of activated lymphoid cells. Enzyme (30 μg of acid phosphatase; 45 μg of hyaluronate lyase) was simultaneously injected into the footpads and thighs, above and below the 'activated' mononuclear cell injection site such that the 'activated' cells could be continually presented with additional enzyme. This technique was repeated at one to two weekly intervals. Four treatments failed to raise antibodies to acid phosphatase (Fig. 1c). However, this method was extremely successful in raising high titre antibodies to hyaluronate lyase (Fig. 1d). Antibody titres rose after only one treatment and after three treatments the neutralizing titre had risen to 2^{-8} (1:256). Resting the rabbit for several months after bleeding resulted in a decrease in antibody titre which was subsequently raised to 2^{-9} (1:512) by a further three treatments.

Neutralization of P. granulosum *lipase and hyaluronate lyase by anti-*P. acnes *exocellular enzyme antisera*

Antisera to *P. acnes* lipase and hyaluronate lyase (Figs 1a and 1d) also neutralize the enzymes produced by *P. granulosum* (see Figs 2 and 3). Anti-*P. acnes* lipase neutralized the *P. granulosum* lipase at a titre of 2^{-4} (1:16), compared with a titre of 2^{-5} (1:32) against *P. acnes* lipase. Anti-*P. acnes* hyaluronate lyase serum neutralized the *P. granulosum* enzyme at a titre of 2^{-7} (1:128), compared with a titre of 2^{-9} (1:512) against the *P. acnes* enzyme. This suggests a serological relationship.

However, the preimmune hyaluronate lyase antiserum neutralized the *P. acnes* enzyme at low titres but not the *P. granulosum* enzyme. In addition, the neutralization curves obtained with both the *P. granulosum* lipase and hyaluronate lyase are complex. This implies that the enzymes produced by the two species are not identical.

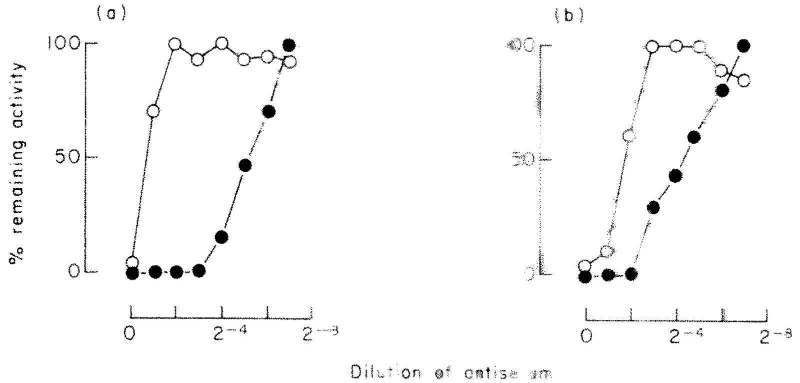

FIGURE 2. Neutralization of (a) *P. acnes* and (b) *P. granulosum* lipases by anti-*P. acnes* lipase antiserum. ○——○, pre-immune serum; and ●——●, immune serum.

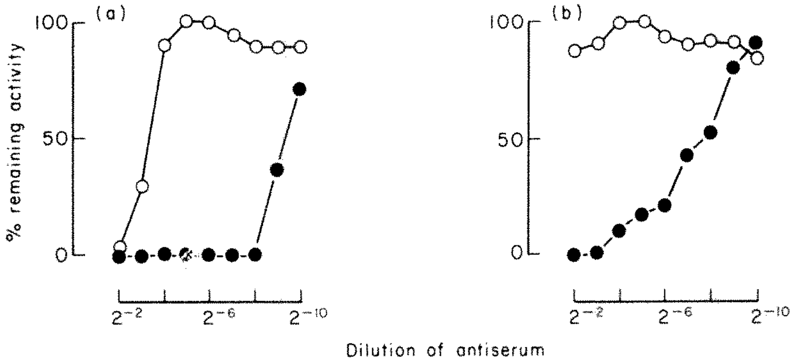

FIGURE 3. Neutralization of (a) *P. acnes* and (b) *P. granulosum* hyaluronate lyases by anti-*P. acnes* hyaluronate lyase antiserum. (a) *P. acnes* hyaluronate lyase; (b) *P. granulosum* hyaluronate lyase; ○——○, pre-immune serum; and ●——●, immune serum.

DISCUSSION

The difficulties encountered in producing laboratory antisera to *P. acnes* exocellular enzymes suggest that, in the rabbit at least, these enzymes are not highly immunogenic. It was possible to produce an antiserum to *P. acnes* lipase using the conventional techniques recommended for soluble proteins (Williams & Chase, 1967) and similar results were obtained using a further two animals.

Conventional immunization procedures failed to produce neutralizing antibodies to *P. acnes* hyaluronate lyase. However, when this enzyme was incubated with rabbit mononuclear cells *in vitro* prior to inoculation with the 'activated' mononuclear cells and simultaneous inoculation of the enzyme in FCA, high-titred antiserum was produced. This indicated that *P. acnes* hyaluronate lyase was immunogenic when presented to the rabbit lymphocytes in this manner. A possible explanation for these results could be that all mammals have a hyaluronate lyase inhibitor in their blood (Dorfman, Ott & Whitney, 1948). This inhibitor, termed antinvasin, inactivates microbial hyaluronate lyase enzymes (Goldberg & Haas, 1947). It is possible that when *P. acnes* hyaluronate lyase was administered to the rabbits i.v. or i.m., that the enzyme was degraded by the rabbit antinvasin and therefore failed to persist as an immunogen. The method used in this study to produce antibodies to *P. acnes* hyaluronate lyase may be useful for producing antibodies to other soluble proteins where conventional immunization procedures have failed.

None of the techniques used in this study were successful in producing antibodies to *P. acnes* acid phosphatase. These results indicate that this enzyme exhibits low immunogenicity in the rabbit. It is well known that rabbits vary in their response to immunogens (Williams & Chase, 1967), however similar results were obtained in four rabbits tested. It may be possible to produce antibodies to this enzyme in a different animal, for example, chickens.

The antisera to *P. acnes* lipase and hyaluronate lyase have been shown to neutralize the lipase and hyaluronate lyase produced by the related skin micro-organism, *P. granulosum*. However, both antisera gave complex neutralization curves with the *P. granulosum* enzymes. These results may be due to the antiserum being a heterogeneous mixture of antibodies with different specificities towards separate sites on the enzyme molecules or could indicate more than one molecular form of the enzymes produced by *P. granulosum*. The results indicate that the *P. granulosum* enzymes are not immunologically identical to the *P. acnes* enzymes. In addition, the preimmune hyaluronate lyase antiserum neutralized the *P. acnes* but not the *P. granulosum* enzyme at low titres. A possible explanation could be that the animal had encountered *P. acnes* hyaluronate lyase but not *P. granulosum* hyaluronate lyase previously.

Whether the normal population or patients with acne become sensitized to the exocellular enzymes which may be produced by *P. acnes in vivo* remains to be determined. The immune status of the normal and acne-affected population with respect to *P. acnes* cells has been well documented (Woldberg *et al.*, 1977; Gowland *et al.*, 1978; Kersey, Sussman & Dahl, 1980; Puhvel, Hoffman & Sternberg, 1966). It may be reasonable to speculate that little sensitization to the enzymes would occur in normal individuals as the enzymes have been shown to exhibit low immunogenicity in at least one mammalian species. However, in patients with inflammatory acne there is an infiltration of lymphocytes into the sites of inflammation in the later stages of the acne process (Kligman, 1974). It is possible that when the lymphocytes encounter the enzymes together with a potent adjuvant (*P. acnes*; Halpern, 1975) sensitization to at least two of the exocellular enzymes could occur.

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The fetal prognosis in pemphigoid gestationis (herpes gestationis)

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SUMMARY

We have studied the infants in fifty pregnancies affected by pemphigoid gestationis (herpes gestationis). There was a significant increase in the frequency of infants that were 'small for dates'. As such infants have a raised mortality and morbidity it follows that in pemphigoid gestationis the fetal prognosis is impaired. In view of this it is essential that patients with pemphigoid gestationis are delivered in maternity units which have facilities for intensive care of the newborn.

There has long been uncertainty over the fetal prognosis in pemphigoid gestationis (PG) (formerly called herpes gestationis) and this has not been dispelled by two recent studies. A literature review of immunofluorescence positive cases indicated that the risk to the fetus was substantial (Lawley, Stingl & Katz, 1978) while a further report found no evidence of increased morbidity or mortality (Shornick *et al.*, 1983). The inconsistency of these studies may stem from the fact that their conclusions were based solely on the fetal outcome which could have varied with the standard of obstetric and perinatal care. A fundamental method of assessing fetal risk is to simply measure the infant weight and compare it with the weight expected for the gestation. This information has not been recorded in previous studies of PG. In our patients documentation of the infants' weights and gestations and calculation of the incidence of 'small for dates' infants has enabled us to clearly define the fetal risk in PG.

METHODS

Our patients were submitted by physicians throughout the United Kingdom. The diagnosis of PG was supported by positive immunofluorescence findings in all but two patients who had PG prior to the introduction of immunofluorescence techniques. In forty-seven cases details of the infants were obtained by one of us (R.C.H.) in interviews with the mothers and examination of the obstetric notes. In the remaining three cases the details were obtained from the referring consultants. In addition to documenting the infant's birth weight and gestation, its development

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at birth was calculated on a standard growth chart (Gairdner & Pearson, 1971). The mode of delivery was recorded. The birth weights, stillbirth rate and infant death rate were compared with the findings in England and Wales during 1981 (*Mortality Statistics 1981: Infant and Perinatal*; Series DH3 no. 4. HMSO, 1982). The frequency of 'small for dates' infants was compared with the findings in 46,703 consecutive births (Thomson, Billewicz & Hytten, 1968). Results were analysed statistically using the binomial test.

The infants were examined for congenital abnormalities and to detect whether their skin was affected by PG. A note was made of the pregnancies in which systemic corticosteroids were administered.

RESULTS

We studied thirty-three patients in whom fifty-four pregnancies were affected by PG. Three pregnancies were terminated surgically at 8 weeks, 1[illegible] weeks and 18 weeks gestation respectively because of the development of PG. A further pregnancy ended in a spontaneous abortion at 12 weeks gestation. Therefore, fifty pregnancies progressed to parturition.

TABLE 1. Pemphigoid gestationis details of the infants

Case	Gravida	Sex	Weight (kg)	Gestation (weeks)	Case	Gravida	Sex	Weight (kg)	Gestation (weeks)
1	9	M	3·18	40	16	4	M	1·79	34*
2	1	M	1·93	40*	17	2	M	4·11	41
	2	F	1·76	38*	18	1	F	3·54	41
	3	F	2·83	40		2	TOP		
3	1	F	1·58	40*	19	3	F	3·72	40
4	2	M	3·24	39	20	2	M	3·46	40
5	8	M	1·94	36*	21	3	F	3·18	40
6	1	M	3·44	39		5	F	3·18	39
	2	M	3·07	38	22	5	M	3·40	40
7	4	F	2·93	35		6	F	2·84	36
	5	F	1·82	39*	23	1	F	2·52	37
	6	M	1·82	38*		2	F	2·27	37*
8	2	M	3·16	41	24	2	M	3·51	30
9	4	M	2·82	40*	25	1	M	3·35	42
10	1	M	3·12	40		2	Spontaneous abortion		
	2	M	3·78	40		3	F	2·92	39
	3	TOP			26	1	F	3·86	42
11	5	M	3·49	38		2	F	2·50	34
12	1	M	2·84	39	27	3	F	2·72	40*
	2	M	3·27	38		4	F	2·1	36*
13	1	M	3·30	40	28	2	M	2·72	32
	2	M	3·30	40		4	M	3·45	38
	3	M	2·95	40	29	7	F	1·44	32
	4	M	3·01	40	30	3	M	1·4	32*
	5	M	2·84	40*	31	4	M	3·32	40
14	4	TOP			32	1	F	2·90	39
15	5	F	2·83	36	33	1	F	3·70	39

TOP = termination of pregnancy
*Small for dates.

TABLE 2. The fetal prognosis in pemphigoid gestationis

	Pemphigoid gestationis	Normal
Low birth weight (< 2·5 kg)	26% (13/50)	7·2%*
Small for dates (below tenth centile)	26% (13/50)	10%†
Stillbirths	0	0·7%*
Infant deaths	8% (4/50)	1·1%*

**Mortality statistics 1981: Infant and perinatal*; Series DH3 no. 4 HMSO (1982).

†Thomson, Billewicz & Hytten, (1968).

Ten infants (20%) were delivered by caesarean section. The indications were fetal distress (5), pre-eclampsia (1), cord prolapse (1), placenta praevia (1), previous caesarean section (1) and in one patient the indication was not clear. In the cases with fetal distress caesarean sections were performed at 32, 34, 36, 36 and 40 weeks respectively. Sixteen infants (32%) required admission to a special care baby unit. Details of the infants' sex, birth weights and gestations are listed in Table 1. There was a significant increase in the frequency of low birth weight (two-tailed, $P = 0{\cdot}00008$) and 'small for dates' infants (two-tailed, $P = 0{\cdot}002$). The infants' deliveries were pre-term (< 37 weeks) in 20% (10/50) of pregnancies. The mean gestation at the onset of PG was 20 weeks in the pregnancies with 'small for dates' infants and 22 weeks in the remaining pregnancies. The delivery of 'small for dates' infants did not appear to correlate with the severity of PG.

There were no stillbirths and four infant deaths (Table 2). Details of the infant deaths are listed in Table 3. Pre-eclampsia occurred in only one pregnancy (case 30). The baby was delivered by caesarean section at 32 weeks because of fetal distress and, although he survived, he had moderately severe cerebral damage. One infant (case 16) was noted to have purpura on the face and trunk following a caesarean section for fetal distress. Investigation revealed severe thrombocytopenia secondary to a maternal anti-platelet antibody. The mother's platelet count was normal and the infant's thrombocytopenia resolved spontaneously by 1 month. Only one

TABLE 3. Infant deaths in pemphigoid gestationis

Case	Gravida	Mother's age	Delivery	Infant's age	Cause of death
7	6	31	ND	6 weeks	Congenital heart disease
24	2	33	ND	4 months	Cot death
28	2	20	ND	2 days	Respiratory distress syndrome
29	7	44	CS	5 days	Edward's syndrome, Ventriculo-septal defect, Respiratory distress syndrome

ND = Normal delivery.
CS = Caesarean section.

infant developed a bullous eruption resembling PG (case 1). The child was born with bullae on the scalp which resolved within 1 week of delivery.

Systemic corticosteroids were administered in 44% (22/50) of pregnancies. In such pregnancies the average birth weight was 2·74 kg and there were 7/22 (32%) 'small for dates' infants. In pregnancies where systemic corticosteroids were not prescribed the average birth weight was 3·00 kg and there were 6/28 (21%) 'small for dates' infants.

DISCUSSION

In the early reports of PG, assessments of the fetal prognosis differed considerably (Downing & Jillson, 1949; Kolodny, 1969). This might have resulted from inconsistency in the diagnosis of PG. When immunofluorescence techniques were introduced and it became possible to define PG more precisely, Lawley *et al.* (1978) reviewed the fetal prognosis in immunofluorescence positive cases. They concluded that the fetal mortality and morbidity were increased. However, Shornick *et al.* (1983) have suggested that literature reviews will be biased towards the more severely affected cases and in their own study of twenty-eight patients they found that the fetal prognosis was normal. Therefore recent studies have failed to dispel the uncertainty that surrounds the fetal prognosis in PG. As the likely outcome for the fetus is one of the most important factors in the management of patients with PG we have examined the birth weights, gestations and frequency of 'small for dates' infants in fifty affected pregnancies in order to clarify the fetal prognosis.

Birth weight is the most important single factor in determining neonatal mortality and morbidity, with infants under 2 kg having a mortality in excess of 10%. It has also been possible to identify a medium risk group (mortality 1·5–10%) in which pre-natal growth has been impaired and the infants are 'small for dates' (< tenth centile) (Behrman, Babson & Lessell, 1971). In addition to their increased mortality, 'small for dates' infants have a raised frequency of long-term mild cerebral dysfunction (Fitzhardinge & Steven, 1972). As our study has demonstrated a significant increase in the frequency of both low birth weight and 'small for dates' infants in PG (Table 2) we have clearly established that the fetal prognosis is impaired. We were unable to predict which pregnancies were particularly at risk as the delivery of 'small for dates' infants did not appear to correlate with either the severity or the early onset of PG.

Despite the increase in infant risk factors the mortality and morbidity which could actually be ascribed to PG in our cases was small. In our fifty infants there were four infant deaths and no stillbirths. Of the four infant deaths it seems likely that three (cases 7, 24, 29) were unrelated to PG. In these three infants the causes of death were congenital heart disease, cot death and Edward's syndrome. Edward's syndrome is a chromosomal disorder (trisomy 18) in which the abnormalities include developmental and mental retardation, and also cardiac malformations. In 30% of cases death occurs in the neonatal period. The disorder is thought to result from non-disjunction during meiosis (Hecht, 1979). This is a problem encountered more frequently with advancing maternal age and therefore its occurrence in our infant was probably related to the mother's age (44 years) rather than her PG. One child sustained cerebral damage (case 30) and this was probably also unrelated to PG but rather to the mother's severe pre-eclampsia.

The explanation for the satisfactory outcome in most of our infants may well have been a result of the good obstetric and perinatal care of our patients. Sixteen (32%) of the babies in our study were admitted to a special care baby unit and without these facilities there would probably have been a considerably greater mortality and morbidity. Our patients also benefitted from close antenatal supervision. This was illustrated by the fact that in five of our cases (10%)

emergency caesarean sections were performed for fetal distress at 32, 34, 36, 36 and 40 weeks respectively.

Infants with bullous lesions resembling PG have occasionally been reported. This has been attributed to passive transfer of the mother's anti-basement membrane zone (BMZ) antibody across the placenta (Chorzelski *et al.*, 1976). We found clinical involvement of the infants to be a very uncommon feature with only one (2%) of our infants affected. It is perhaps surprising that neonates are so rarely affected as the anti-BMZ antibody is known to be an IgG (Jordon *et al.*, 1976; Katz, Hertz & Yaoita, 1976) and IgG usually crosses the placenta.

There have been two reports of an association between PG and the HLA antigen DR3 (Shornick, Stastny & Gilliam, 1981; Holmes *et al.*, 1983). A raised frequency of HLA DR3 has also been reported in several auto-immune disorders (Svejgaard *et al.*, 1981) and it has been proposed that DR3 is a marker of high immune responsiveness (Batchelor, 1980). This would suggest that patients with HG might have a predisposition to develop other auto-immune disorders and, in support of this, we have reported a possible association with Graves' disease (Holmes & Black, 1980). The propensity of patients with PG to develop other auto-immune problems is relevant to the fetal prognosis. This was illustrated by case 16 where the infant was born with thrombocytopenic purpura. Investigations revealed that the thrombocytopenia was secondary to an anti-platelet antibody which had been produced by the mother, although the antibody had not affected the mother's own platelet count.

As systemic corticosteroids are the mainstay of treatment for PG it is important to consider whether this treatment itself can effect the fetal outcome. Schatz *et al.* (1975) studied seventy pregnancies in fifty-five asthmatic patients where prednisolone had been given in a dosage of 2·5 to 20 mg daily (mean 8 mg). They concluded that there was no increase in fetal complications. In our study of PG 44% (22/50) of patients received systemic corticosteroids during pregnancy and there were no striking differences in birth weight and frequency of 'small for dates' infants between these patients and those who did not receive corticosteroids.

In conclusion we have demonstrated an increase in low birth weight and 'small for dates' infants in PG and therefore an increase in fetal risk. However, with good obstetric and perinatal care the outcome in the majority of pregnancies was satisfactory. The mortality that did occur in our patients was mostly related to coincidental problems. The administration of corticosteroids during pregnancy did not appear to effect the fetal outcome adversely.

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Pharmacology and Treatment

The effect of terfenadine on dermographic wealing

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SUMMARY

A double-blind comparison of terfenadine with placebo showed a reduction in potency of measured dermographic force–response of 67%, which is similar to that for inhibition of histamine weals by terfenadine. This and the parallel displacement of the force–response curves indicates that histamine is the main cause of dermographic wealing. Dermographic threshold and itch change together and appear to be the main determinant of the patients' subjective response. Dermographism relapsed towards its pre-treatment state during the 3 days after treatment was stopped. There was a small decrease in effect after administration of 60 mg b.d. for 47–84 days and a further small increase in response when the dose was increased to 120 mg t.d.s., but neither change was significant. None of the patients had a sedative response to the drug and terfenadine should prove useful in the treatment of wealing disorders.

Terfenadine is an H1 receptor antagonist without soporific activity in doses which produce significant inhibition of histamine wealing (Huther *et al.*, 1977; Reinberg *et al.*, 1978; Nicholson & Stone, 1982; Ford, Krause & Shuster, in preparation). We therefore studied its effect on patients with dermographism both by objective measurement and by subjective self-assessment.

METHODS

The investigation was in two parts: (1) a double-blind study comparing short-term terfenadine with placebo, (2) an open study of prolonged effect and dose response.

1. Double-blind short-term study

We selected ten consecutive patients presenting with dermographism who had had the disorder constantly for at least 2 months, but were otherwise healthy, and were aged 16 years or more. All were females, aged 18–38 years and most were poorly controlled by a variety of H1 receptor antagonists. They were asked to stop all treatment for 3 days after which they were given, on a double-blind basis, treatment A for 3 days, followed by treatment B for 3 days; they were seen again 3 days after stopping treatment B. Unknown to both the patients and the investigator in direct contact with them, treatment A was placebo tablets and treatment B was terfenadine (Triludan®) 60 mg tablets – both identical in appearance and similarly taken twice daily.

Objective assessment. Wealing studies were performed before treatment, on the last day of treatments A (placebo) and B (terfenadine), 4–6 h after taking the tablets, and at the end of a 3-day period without any treatment. The principle of the method was to measure shifts in the force/weal stimulus–response curve produced by treatment (Cook & Shuster, 1983). Weals were induced with a spring-loaded stylus the force on which could be varied by linear compression of the spring (James & Warin, 1969; Kirby *et al.*, 1971). The skin of the back was chosen, on each side of the vertebral column between T2 and T12, as preliminary observations suggested that this site was optimal for weal reproducibility and size. We selected stylus pressures corresponding to the linear compression calibrations of the stylus spring in mm, and measured the actual force produced at the stylus tip for each calibration. Weals were induced by moving the stylus up and down the skin surface three times using a metal guide and the wealing produced by each force was measured 10 min later from the mean of three diameters, randomly chosen along the weals, marked by a pen and measured with a ruler. The variability in the three measurements of each weal was found to be 8·6% ± 0·5 s.e. (n = 100 weals from ten subjects). For each force increment, weals were produced in duplicate symmetrically on each side of the vertebral column. There was no significant difference between duplicate readings (R/L variability mean 9·2% ± 0·6 s.e., n = 100 weals from ten subjects) and the mean of both readings was taken as the response to each stylus force.

Subjective assessment. All patients made a daily record of the severity of itching and drowsiness using 10-cm analogue scales on a diary card.

2. Open study of prolonged administration dose response

(a) After the double-blind study was completed all the patients were maintained on terfenadine 60 mg b.d. for 47–84 (mean 64) days of treatment, when the objective and subjective assessments were repeated on seven of the original ten patients (one patient failed to re-attend, one was having a cholecystectomy, and one had stopped taking the terfenadine and was no longer dermographic). (b) The dose of terfenadine was then increased to 120 mg t.d.s. and the patients were assessed again on the 7th day of treatment, 4–6 h after taking the tablets.

Weal threshold. The minimal force required to produce a weal was recorded for each patient both as calculated load on the stylus tip and minimum compression of the stylus spring. However the calibration of the instrument does not permit measurement of threshold $< 28\cdot6$ g/mm^2 which corresponds to a spring compression of zero mm.

RESULTS

1. Double-blind short-term study

All ten patients completed the study.

Force/weal stimulus response. Weal diameter increased with the dermographic stimulus whether calculated as stylus load (Fig. 1) or as linear spring compression (Fig. 2), although the fit was slightly better for the former. This relationship was observed in the pre-treatment, placebo and post-treatment studies. During terfenadine treatment the curve became non-linear

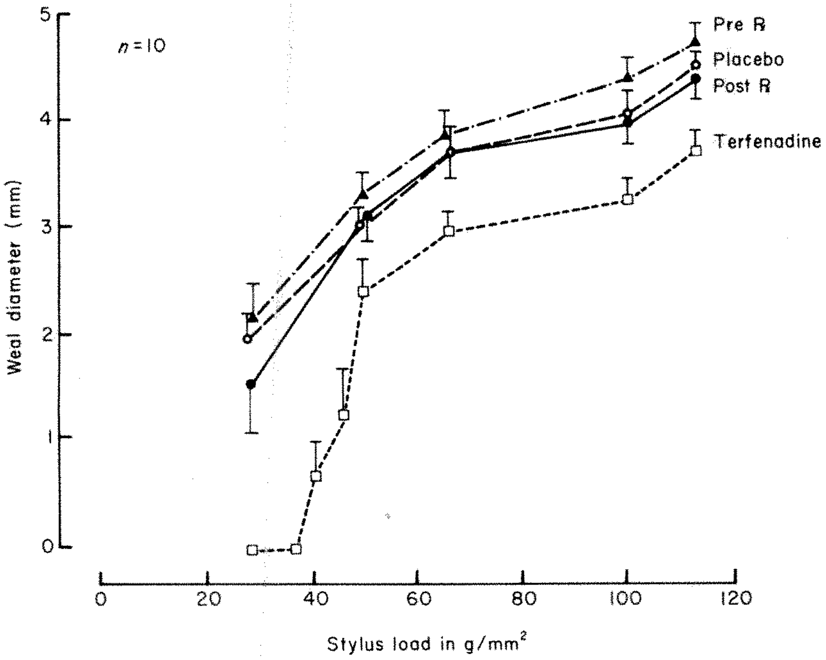

FIGURE 1. Stylus force–weal response curve before treatment (▲), during placebo (○), 3 days after treatment (●) and on the third day of treatment with terfenadine 60 mg b.d. (□).

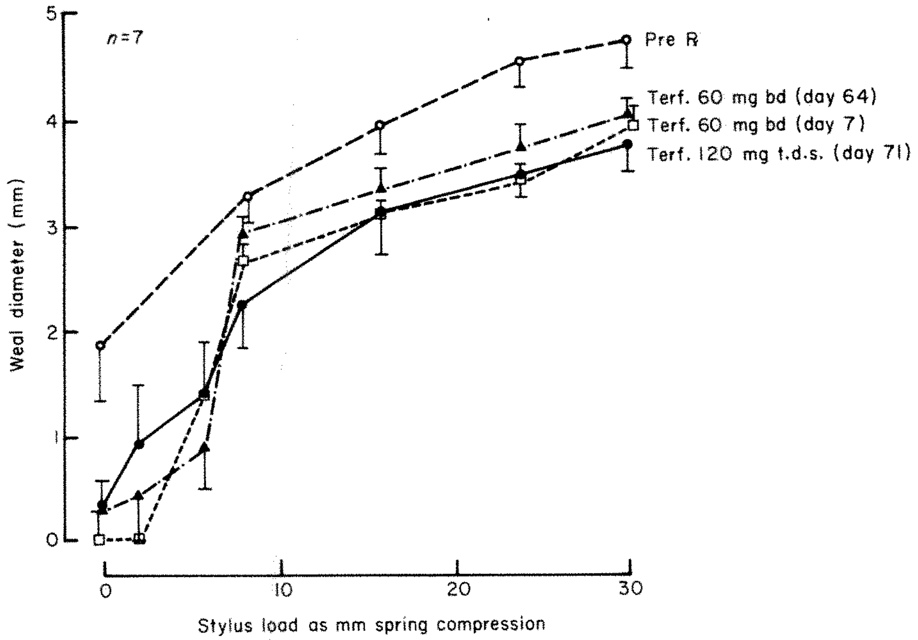

FIGURE 2. Stylus force–weal response curve before and during terfenadine treatment. Stylus force is expressed as spring compression to show the results are similar to those expressed as calculated load on the stylus tip in Fig. 1.

below a stylus force of 50 g/mm^2 due to the zero values in patients with a change in weal threshold, although if patients below the threshold are excluded the curve remained approximately linear. Calculations are therefore based on the linear component above the threshold. The results computed from regression analysis show a slight but insignificant shift of dose response for placebo and a significant shift after terfenadine which reduced the weal force potency by 67%. Three days after stopping terfenadine the force/weal response had almost returned to pre-treatment levels (Fig. 1).

Weal threshold. The change in weal threshold is shown as a mean in Table 1 and as the percentage of patients wealing with each spring compression load in Fig. 3. Before treatment the lowest stylus forces used were above the weal-force threshold in all but two patients and the threshold was passed in them by the second lowest force used. Placebo had no effect on weal threshold. After terfenadine the elevation of the dermographic weal-force threshold was even

TABLE 1. The effect of terfenadine on weal threshold (expressed both as stylus load and compression of stylus spring) in ten patients with dermographism

	Pre-treatment	3rd day of treatment	3 days after treatment
Mean weal threshold ± s.e. (stylus load in g/mm^2)	30·8 ± 1·5	44·9 ± 1·1	35·0 ± 2·9
Spring compression in mm	0·3 ± 0·2	2·8 ± 0·3	1·15 ± 0·5

N.B. The lower limit of threshold measurement is 28·6 g/mm^2 which corresponds to zero compression of the spring.

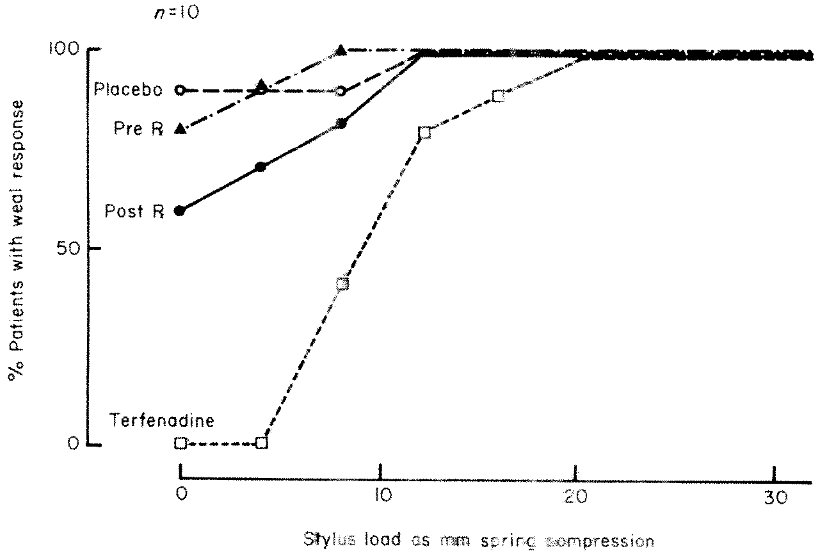

FIGURE 3. Weal threshold from data in Fig. 1 expressed as mm spring compression.

more dramatic than the change in weal–force dose response, none of the patients wealing with a stylus force of 27 g/mm² compared with 90% before treatment, all patients crossing the threshold of response with a stylus force of 58 g/mm² compared with a force of 27 g/mm² for the same group before treatment. These differences are highly significant ($P < 0.001$). There was still some elevation of weal threshold 3 days after stopping terfenadine ($P < 0.01$).

Comparison of weal diameters and subjective assessment of itch and dermographism. No correlation was found between weal diameters measured before treatment and the patient's own assessment of severity of itch (10-cm line) nor with the clinician's own assessment of severity (1–4 clinical rating) at entry to the study. During treatment with terfenadine changes in itch were greater than inhibition of the weal/force response and corresponded best to the changes in dermographic weal threshold.

Drowsiness. Terfenadine did not produce drowsiness (10-cm linear analogue scale) in any of the patients but drowsiness occurred as a placebo response in two patients. One patient was extremely drowsy (10-cm on linear analogue scale) on the first placebo day and another was also extremely drowsy (10 cm) throughout the placebo days and on the first 2 days of terfenadine administration but not on the third day (0 cm).

2. *Open study of prolonged administration*

Dermographism. (a) Amelioration of the dermographic force–weal response and weal threshold after 47 to 84 days terfenadine 60 mg b.d. was similar to that found after 3 days (Fig. 2) although the response was slightly but not significantly less. (b) When the dose of terfenadine was then increased to 120 mg t.d.s. inhibition of the weal response increased slightly and there was some shift in the dermographic threshold but these differences were not significant.

Itching. Amelioration of itching was similar to the changes in dermographism. Thus itch was slightly but not significantly less after 3 days of 60 mg b.d. (median 0 cm) than after 47–84 days (median 4 cm) and decreased again with 120 mg t.d.s. (median 2 cm) ($P = 0.05$).

Sedation. No sedation was noted with 60 mg b.d. for 47–84 days followed by 120 mg t.d.s. for 7 days.

DISCUSSION

The present study shows that terfenadine greatly reduces dermographic wealing and itch without subjective somnolence. The study also confirms the reproducibility of the method used to induce dermographic wealing and its suitability for the quantitative assay of drug response. There was however, no correlation between the objective measurement of susceptibility to dermographic wealing and the clinical assessment of severity taken from the history and the patient's estimate of itch. Thus subjective assessment of dermographism can only be used as a comparative measure of response in a particular individual and not for comparison between individuals, whereas objective measurement of wealing can be used for both. We did not detect any effect of placebo on the histamine weal response in a previous study (Cook & Shuster, 1980) and there were no alterations in weal threshold and itch after placebo in the present study: we therefore suspect that the slight depression of the weal–force curves was due to measurement bias. The excellent correlation between the patient's assessment of itch and the shift in weal threshold with terfenadine suggests that the patients' concern with dermographism is more related to threshold responses, e.g. the response to minor scratch, than to the more dramatic weals which are used to demonstrate the disorder clinically. This accords well with the course of the condition, the patients initially experiencing irritation rather than wealing, the more obvious weals arising only after they indulge an increasing itch with an increasingly appropriate scratch.

The findings show that with a dose of 60 mg terfenadine twice daily, itch and the wealing response to low forces was abolished in all patients. In addition to the rise in dermographic weal threshold the dermographic force–response curve was shifted in parallel to the right giving a depression of dermographic potency of the order of 47%. This is comparable to the degree of suppression of histamine weals by the same dose of terfenadine (Huther *et al.*, 1977; Reinberg *et al.*, 1978; Ford *et al.*, in preparation). The comparable magnitude of effect and the parallel shift of the force dose–response curve to the right which is classically that of competitive inhibition at a common receptor (Arunlakshana & Schild, 1959) or post-receptor site (Carter & Shuster, 1982) is strong presumptive evidence that dermographic wealing is indeed due to histamine release (Greaves & Sondergaard, 1970).

Some effect of terfenadine was still apparent 72 h after stopping the drug though it was less than at 24 h. More important is the observation that the drug was still effective after 47–84 days administration in a clinically effective dose of 60 mg b.d. The question of whether the drug was equally effective requires further study because the small changes in degree of amelioration of itch and wealing could indicate altered metabolism of the drug, a change in H1 receptor population or some other cause of antagonist tachyphylaxis. In these circumstances it would be important to know whether increasing the dose of terfenadine could restore the weal inhibitory effect. A dose of 60 mg terfenadine b.d. appears to be maximal for inhibition of the histamine weal in acute studies (Huther *et al.*, 1977; Reinberg *et al.*, 1978) but the dose response in pathological situations and after prolonged administration is not yet established. Although inhibition of itch and dermographism was only slightly greater when the dose of terfenadine was increased to 120 mg t.d.s., because the study was open we cannot exclude observer and patient bias, and a placebo-controlled double-blind study will be required to define the maximum therapeutic dose in dermographism and other disorders. In practical terms, until these questions are answered, 60 mg b.d. appears to be an adequate therapeutic dose, but it would be worth increasing it if the response appears to be inadequate or the condition relapses during treatment.

Although on a molar basis terfenadine is not a particularly potent H1 receptor antagonist compared with many of the others available, this is irrelevant to clinical efficacy which is determined by a high therapeutic ratio. The main unwanted effect of H1 antihistamines is sedation and none of our patients were drowsy even after 47–84 days of terfenadine 60 mg b.d. followed by a week of 120 mg t.d.s. whereas chlorpheniramine, a commonly used antihistamine is usually sedative even in doses which achieve less inhibition of wealing (Cook & Shuster, 1983). Since this study was completed we have found similar results with astemizole, another non-sedative peripheral H1 receptor antagonist (Krause & Shuster, in preparation). Terfenadine and astemizole are the first of a new family of H1 receptor antagonists in that they achieve clinically significant H1 receptor blockade without sedation (Nicholson & Stone, 1982); if usage proves them to be safe they are likely to replace other antihistamines when a peripheral H1 antagonistic action is required.

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Application of controlled high dose rates in UV-B phototherapy for psoriasis

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SUMMARY

The use of a cabin fitted with Sylvania UV-21 tubes, which have a very high output of UV-B radiation, made it possible to treat psoriasis patients with a maximal exposure time of 5 minutes per session. An exposure-control unit was used to measure and regulate the applied dose to prevent overdosage. The clinical results obtained with two comparable groups of psoriasis patients treated with Sylvania UV-6 or UV-21 tubes were equal.

UV-B phototherapy with various radiation sources has attracted increasing interest in the last few years (Tronnier & Heidbüchel, 1976; Hölzle, 1980; Adrian *et al.*, 1981; Boer, Schothorst & Suurmond, 1981; Larkö, 1982). The results have been reported to be either equal to or only slightly inferior to those obtained with PUVA (van Weelden, Young & van der Leun, 1980; Kenicer *et al.*, 1981; Parrish, 1981; Vella Briffa *et al.*, 1981; Boer *et al.*, 1983). UV-B therapy has several advantages over PUVA, including the absence of nausea, much less pruritus, no photosensitivity on the day of treatment, no need for contraception, shorter irradiation times, and possibly fewer long-term oncogenic effects, although this last remains to be proved (van Weelden *et al.*, 1980; Larkö, 1982). Further improvement of UV-B phototherapy might be achieved by reducing UV penetration into normal skin and increasing it in the lesions (Anderson, Le Vine & Parrish, 1980; Boer *et al.*, 1982; Boer *et al.*, 1983), minor alterations in the emission spectrum of the lamps, and shortening of the exposure times. The present study dealt with the exposure time.

In previous studies we used walk-in booths, fitted with either Philips TL-12 or Sylvania UV-6 lamps for whole-body irradiation, and these were equally effective (Boer *et al.*, 1983). Both, however, required long irradiation times during the clearing phase, i.e. up to 30 minutes, which means that the patient must remain standing for what is quite a long time, particularly for older people. This situation led us to test the Sylvania UV-21 lamp, which produces about four times more light energy in the UV-B region than the UV-6 lamp does. Moreover, the UV-21 lamp has a wider erythematogenic spectral distribution, which seemed likely to permit treatment of psoriasis patients at a high dose rate and very short irradiation times.

In view of the very short irradiation times (ranging from a few seconds to a few minutes), the need for a highly reliable method for dosage control became even greater than for the Sylvania UV-6 lamps, since with the UV-21 a slight overdosage is sufficient to cause severe burns.

The present paper reports the results of a study performed to compare treatment with UV-6 and UV-21 lamps. In addition a new method of dose control is described.

METHOD

Light sources

We used a Waldmann 1000 light booth (Waldmann, Schwenningen, West Germany) with twenty-eight Sylvania F75/85 W UV-6 fluorescent lamps and one with twenty-eight Sylvania F75/85 W UV-21 lamps as irradiation sources, for estimation of the minimal erythema dose (MED) and treatment of psoriasis patients.

Determination of spectral energy distribution

The energy spectra of the UV-6 and the UV-21 lamps were measured with a Cary double-beam Spectrophotometer (Model 14), reciprocal dispersion 0·7 nm. The relative values obtained are shown in Fig. 1.

Determination of UV light intensity

Light intensities were measured with a spectroradiometer with detector SEE 400 SIN 1008 and filter WBS 320 (½ power bandwidth 257–392 nm) covering the entire UV area of the lamps. The sensor was placed at a height of 1 m and at a distance of 20 cm from the TL tubes. According to the energy spectra of the UV-6 and UV-21 lamps (Fig. 1), the measured values were multiplied by either 0·51 or 0·48, respectively, to calculate the energy in the UV-B (280–320 nm) region.

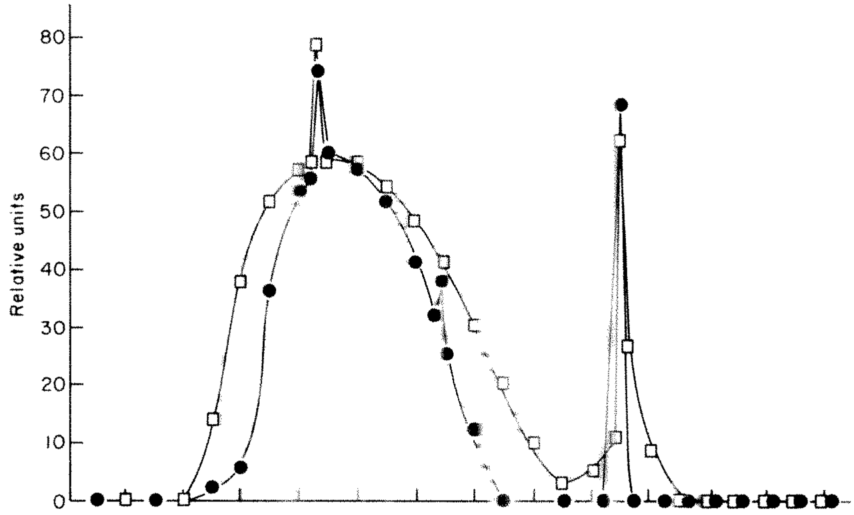

FIGURE 1. Spectral distribution of UV-6 and UV-21 lamps, in relative units. (●——●) UV-6, (□——□) UV-21. The energy in the UV-B (280–320 nm) region of the UV-6 and UV-21 lamps amounts respectively to 51% and 48% of the total UV light energy (280–400 nm).

The light intensity in the UV-6 cabin was 0·61 mW/cm² UV-B and in the UV-21 cabin 2·6 mW/cm².

Light-dose control

The light booth with the UV-21 lamps is equipped with an exposure control unit manufactured by International Light (IL 844/CM 268) which is connected with the detector and filter mentioned above. This detector is mounted behind one of the TL tubes. The measured values are compared and checked with the IL 700 radiometer. The detector continuously monitors the instantaneous irradiance level. The prescribed dose of UV radiation in mJ/cm² is directly set with a digital switch on the control unit, the settings ranging from 5 to 1000 mJ UV-B/cm². The lamps are turned off automatically when the required dose is obtained. In both types of light booth the irradiation time is controlled by a timer (1–10 min). In addition, an assistant checks the exposure time with a stopwatch.

Light tests

Light tests were performed on one of the patient's buttocks. The diameter of each test area was 1·5 cm. The applied light energy in the UV-6 booth ranged from 35 to 210 mJ UV-B/cm² and in the UV-21 booth from 45 to 270 mJ/cm². For patients previously irradiated in the UV-6 booth for maintenance therapy, the tests were performed with light doses ranging from 90 to 550 mJ UV-B/cm², before they were treated in the UV-21 booth. The response was evaluated 24 hours later.

Patients and grading

Initial phase of the treatment. During a period of 14 months thirty-five patients and during a 12-month period thirty-three patients with psoriasis of equal severity were treated in the UV-6 and UV-21 cabins, respectively. Table 1 gives the relevant data of the patients at the start of the study.

TABLE 1. Relevant data for the patients at the start of the study

	UV-6 cabin	UV-21 cabin
No. of patients	35	33
Sex distribution (male/female)	1·5/1	0·65/1
Mean age (yr)	44·1	34·8
Mean duration of psoriasis (yr)	21·8	11.2
Mean extent of psoriasis ± s.d. (% body surface)	32·6 ± 14.3	35·3 ± 15·6
Type of psoriasis (%)		
Plaque	94·3	93·9
Guttate	5·7	6·1
Erythroderma	0	0
Skin type (%)		
I	0	0
II	14·0	6·0
III	68·6	75·8
IV	11·4	18·2
V	5·7	0

The criterion for UV-B therapy was involvement of at least 10% of the body surface. All oral medication known to affect psoriasis was discontinued 1 month before the start of the initial treatment and none was taken throughout the period of the study. Furthermore, no topical medication was permitted except 2% salicylic acid in white petrolatum for all body areas except the scalp.

Patient evaluation was scheduled prior to the first irradiation treatment and every 2 weeks during the clearing phase. At that time the percentage of involvement was estimated relative to the original status of the lesions. The therapeutic effect was scored as follows: 0–49%; 50–79%; 80–94% and 95–100% clearance of lesions. Further details of this treatment regimen have been reported elsewhere (Boer, 1982).

Maintenance phase of the treatment. Patients who showed at least 80% improvement (relative to the status at the start of the initial-phase treatment comprising at most thirty-five sessions) were put on a standard maintenance schedule. An attempt was made to reduce treatment frequencies to one irradiation a week or less (Boer, 1982). Patients were examined every month.

A group of forty-six patients already receiving maintenance treatment in the UV-6 booth were switched over to the UV-21 booth. Throughout the whole-body irradiation with the UV-21 lamps, the patients received a sub-MED dose, i.e., the highest dose in the test series that did not produce visible erythema.

TABLE 2. Results of clearing treatment in patients treated with UV-6 and UV-21 fluorescent lamps

	UV-6			UV-21		
No. of patients treated	35			33		
No. of patients cleared	29 (80%)			29 (88%)		
Reasons for termination of treatment						
Failed to clear	4 (11%)			3 (9%)		
Side-effects	1 (3%)			1 (3%)		
Other reasons	1 (3%)			—		
	Mean	s.d.	Range	Mean	s.d.	Range
No. of treatments for clearing (four treatments a week, n = 29)	25	5	8–[illegible]	28	4	17–35
Amount of first dose (log mJ UV-B/cm²) (n = 29)	1.90	0.27	1·56–[illegible]·47	1·75	0·18	1·38–2·15
Exposure time first dose (min) (n = 29)	2·6	1·8	1–[illegible]	0·44	0·2	0·15–0·92
Amount of last dose (log mJ UV-B/cm²) (n = 29)	2·84	0·17	2·3–[illegible]	2·59	0·18	2·20–2·87
Exposure time last dose (min) (n = 29)	20·30	9	5·6–[illegible]	2·68	1	1·0–4.3
Improvement of lesions (%) (n = 29)	88	7	80–[illegible]	91	6	80–100

RESULTS

The dosage of UV radiation in the cabin with Sylvania UV-21 tubes controlled as described above permits the administration of doses ranging from 5 mJ/cm^2 (for approximately 2 sec) to 1000 mJ/cm^2 (6·5 min). During the 14 months of daily use of this booth, no serious overdosage occurred.

As could be expected from the spectral energy distribution (Fig. 1) much less energy was required for one MED in the booth with UV-21 lamps than in the one with UV-6 lamps.

We determined the MED values of three psoriasis patients in both the UV-6 and UV-21 light booths. The amounts of UV-B energy needed in the UV-6 booth were 268, 247 and 206 mJ, and 144 mJ UV-B was the MED for the UV-21 lamps in all three patients.

Table 2 shows the results for the UV-6 group (thirty-five patients) and a comparable UV-21 group (thirty-three patients). The total number of treatments required to achieve clearing and the percentage of improvement of lesions were similar for both groups. The first dose given to the patients treated in the UV-21 light booth (log dose mJ UV-B/cm^2 = 1·75) was significantly lower ($P < 0·015$) than used in the UV-6 booth (log mJ UV-B/cm^2 = 1·90). The same holds for the last dose: the log dose mJ UV-B/cm^2 was 2·59 in the UV-21 light booth and 2·84 in the UV-6 booth. The first of these values is significantly lower ($P = 0·000001$). The reasons for discontinuation of treatment with the UV-6 or UV-21 lamps did not differ significantly.

Fig. 2 shows that the mean amount of UV-B light energy needed for patients on maintenance therapy was about 30% of the amount for the UV-6 light booth. However, some patients needed more and others less than 30%. Because the UV-B light intensity in the UV-21 cabin was much higher (2·6 mW/cm^2), the irradiation time for the same group of patients after switching to the UV-21 cabin was reduced to approximately 10% of the time previously required (Fig. 3).

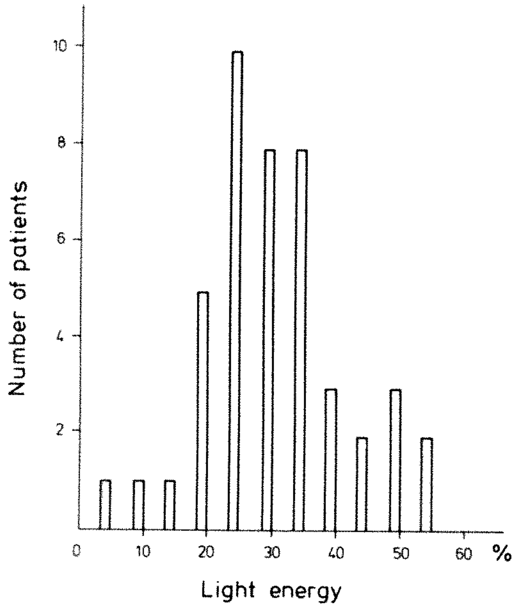

FIGURE 2. Percentage UV-B energy requirement of forty-six patients receiving maintenance treatment after being switched from the UVB-6 to the UV-21 cabin.

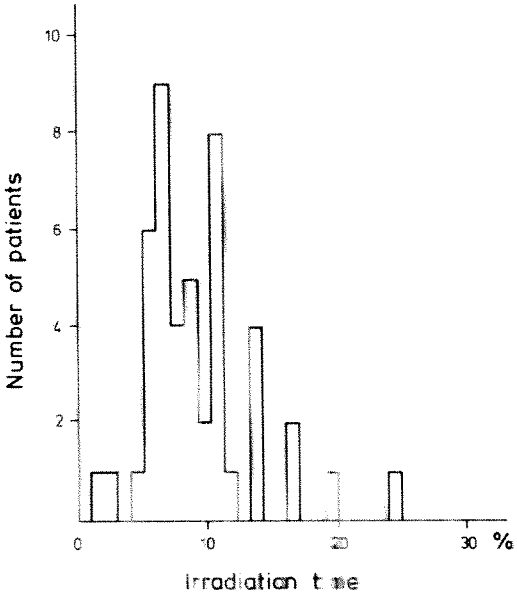

FIGURE 3. The required irradiation time (%) of forty-six patients receiving maintenance treatment after being switched from the UV-6 to the UV-21 cabin.

DISCUSSION

Although many types of irradiation equipment are used for UV-B phototherapy (Pullman, Wichmann & Steigleder, 1978; Hölzle, 1980; Adrian et al., 1981; Boer et al., 1981, 1982), some improvement in the lamps used is possible. In the first place it is very important to determine both the quantity and quality of the applied light doses. The total doses and spectral distribution are extremely important in relation to the induction of skin cancer (Larkö, 1982). Light measurements are, moreover, important because corrections must be applied for decreasing output of UV-B tubes with time of use (Hölzle, 1980). In addition, it can be useful for research purposes to know the spectral distribution (Parrish & Jaenicke, 1981).

To exclude misleading and incomplete spectral distribution values, it is preferable to obtain these values from a non-commercial institute.

With the described booth with the UV-21 lamps it was possible to reduce the irradiation time to 5 min, which is much shorter than the times (20–40 min) mentioned in the literature (Pullman *et al.*, 1978; Hölzle, 1980; Adrian *et al.*, 1981; Boer *et al.*, 1981, 1982). At the high dose rate used, the required dose-control unit seemed to be adequate, but studies on further improvement by the use of additional electronic instrumentation to exclude human errors as much as possible are in progress.

The very short therapeutic irradiation times permitted by the UV-21 booth can be explained by two factors. First, the light intensity per cm^2 in the UV-21 booth is about four times higher than in the UV-6 cabin. But the difference between the spectral composition (Fig. 1) of the two types of tubes is also important (Kenicer *et al.*, 1981). An important difference between the spectral output of the UV-6 and the UV-21 is that the latter lamp emits 11·4% of its UV-B in the region between 295 and 300 nm whereas the UV-6 emits no light in this area. These wavelengths (295–300) are strongly erythemogenic (Cripps & Ramsay, 1970), but therapeutic as well

(Parrish & Jaenicke, 1981). It is also important that the UV-21 emits only a minimal amount of light at wavelengths < 295 nm. According to Parrish & Jaenicke (1981), these wavelengths are erythemogenic but not therapeutic and, if present in broad-band sources, would reduce the efficacy of these light sources.

Patients on maintenance treatment who were switched from the cabin with UV-6 lamps to the cabin with UV-21 lamps proved to be very sensitive to the light emitted by the latter. As shown in Fig. 2, they could tolerate only about 30% of the light energy applied in the UV-6 cabin. This low percentage too can be explained by the different energy composition.

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Case Reports

A case of Pagetoid reticulosis bearing the T cytotoxic suppressor surface marker on the lymphoid infiltrate: further evidence that Pagetoid reticulosis is not a variant of mycosis fungoides

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SUMMARY

We describe a patient with pagetoid reticulosis in whom the lymphocytic infiltrate bore the surface membrane marker of the cytotoxic/suppressor subset. This is further evidence that Pagetoid reticulosis is not a variant of mycosis fungoides.

Pagetoid reticulosis was first described by Woringer & Kolopp (1939). The index case was a boy of 13 who had a lesion on his forearm which slowly extended over a 6-year period. No additional lesions appeared on other body sites. Histological examination revealed an acanthotic epidermis colonized by atypical lymphocytes. Since this report around 40 cases have been described in the world literature and the condition has been considered to be related to, but distinct from, mycosis fungoides. The distinguishing clinical features are its relatively localized distribution, the fact that the lesion, although it may extend locally, remains solitary, and the good response of individual lesions to radiotherapy.

In the past 10 years three groups of workers have reported the presence of T lymphocytes (Braun-Falco, Marghescu & Wolff, 1973), histiocytic cells (Chu & MacDonald, 1980) and Langerhans cells (Geerts, Kaiserling & Kint, 1982) respectively in the epidermal infiltrate. We here present evidence that the bulk of the T lymphocyte infiltrate in this condition is composed of T cells which bear the surface marker of the T suppressor/cytotoxic subset. This is in contrast to the bulk of lymphocytes in plaques of mycosis fungoides which bear the T helper marker.

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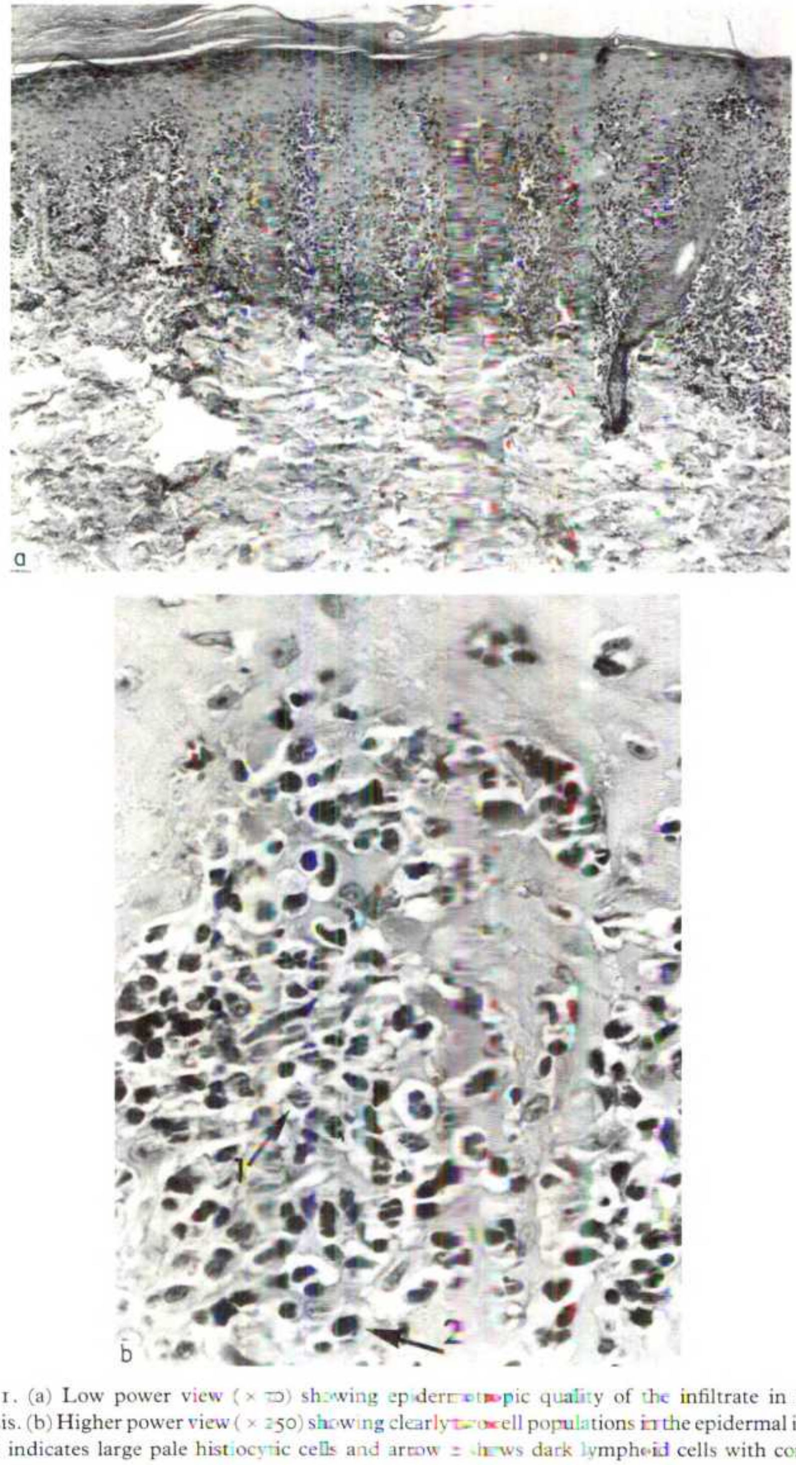

FIGURE 1. (a) Low power view (× 10) showing epidermotropic quality of the infiltrate in Pagetoid reticulosis. (b) Higher power view (× 250) showing clearly two cell populations in the epidermal infiltrate. Arrow 1 indicates large pale histiocytic cells and arrow 2 shows dark lymphoid cells with convoluted nuclei.

CASE REPORT

A male aged 21 years developed a slowly expanding plaque on the dorsum of his right foot and gave a history of trauma prior to the development of the lesion. As the lesion expanded laterally it developed an arcuate margin. Biopsy of the lesion 3 years after its first appearance revealed an acanthotic epidermis colonized by a mixed cell population. At light microscopy level two distinct cell populations were apparent (Fig. 1), namely small lymphocytes and larger histiocytic cells with pale nuclei and a low nuclear–cytoplasmic ratio. Both cell populations exhibited striking epidermotropism and the basal layer of the epidermis was almost completely effaced, giving the histological features a somewhat lichenoid pattern. The underlying dermis, although containing a few infiltrating cells, was much less grossly involved than the overlying epidermis.

T-cell subsets

For identification of T-cell subsets, biopsy tissue was snap-frozen, cut at 4 μm, air-dried, fixed in acetone, and treated with 7·5% H_2O_2 to remove endogenous peroxidase. It was then overlaid with monoclonal antibodies OKT11 (pan T lymphocytes diluted 1:100), OKT4 (helper/inducer lymphocytes diluted 1:50), OKT8 (cytotoxic/suppressor lymphocytes diluted 1:100) and NAI 34 (Langerhans cells and mature thymocytes diluted 1:1000). After 1 h incubation the sections were washed and then reacted with peroxidase-conjugated rabbit anti-mouse antiserum (DAKO, diluted 1:20) for 45 mins. Reaction product was revealed with diaminobenzidine (0·03% in 0·01 M Tris, pH 7·6, with 0·01% H_2O_2 for 5 min). Sections were then lightly counter-stained with haematoxylin, dehydrated and mounted with piccolyte resin (Mackie & Turbitt, 1982).

Histiocytic cells

For identification of the histiocytic cells, paraffin sections were taken to water, treated with methanol with 0·2% HCl and 0·1% H_2O_2 to remove endogenous peroxidase, then incubated sequentially with relevant antibody (lysozyme, α_1 anti-trypsin or α_1 anti-chymotrypsin, 1:100 dilution), swine anti-rabbit IgG 1:100, then peroxidase/anti-peroxidase complex (PAP) 1:100 (all obtained from DAKO). Bound peroxidase enzyme was revealed by reaction with diaminobenzidine as described above. (Papadimitriou, Stein & Papacharalampous, 1980). Appropriate positive and negative controls were used in both immunopathological procedures.

RESULTS

The sections showed a dense infiltrate of lymphocytic cells which bore both the OKT11 and the OKT8 marker in the epidermis (Figs 2 and 3). These cells all showed clear membrane marker staining. In contrast, less than 10% of the lymphocytic population stained with the OKT4 marker. The NAI 34 marker showed only staining of a small number of dendritic cells in the epidermis, having the appearance of a normal population of Langerhans cells for the site concerned. There was not an excessive number of cells stained by this marker.

The bulk of the histiocytic cells stained only with antibodies directed against lysozyme and did not stain with antibodies directed against α_1 anti-trypsin and α_1 anti-chymotrypsin. This pattern of one or two negative results with three antibodies used in this study is not uncommon in our experience.

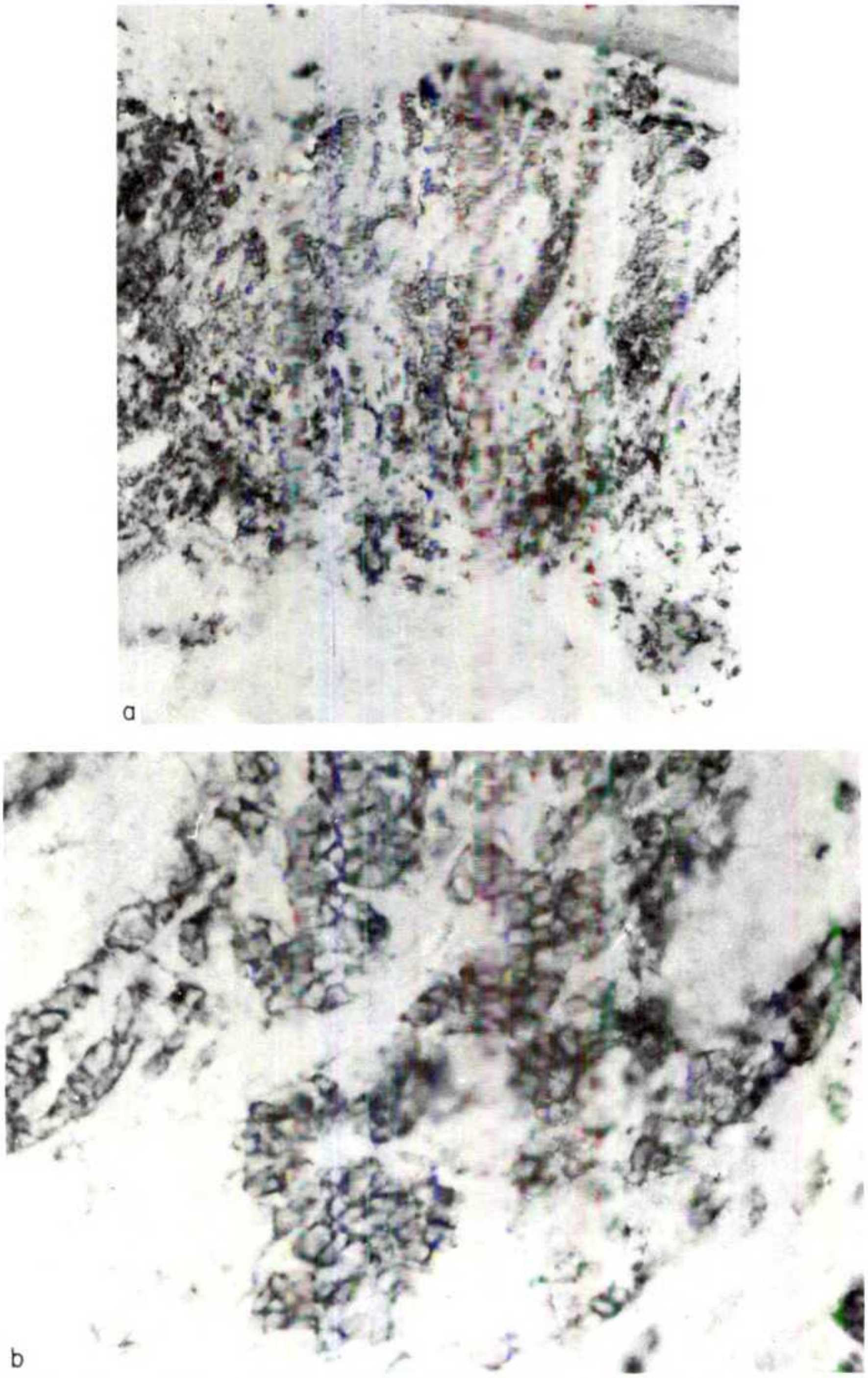

FIGURE 2. (a) Frozen section stained with antibody OKT1 which stains all T lymphocytes. (b) Close-up of frozen section adjacent to 2a stained with OKT8 which reacts with T suppressor/cytotoxic lymphocytes.

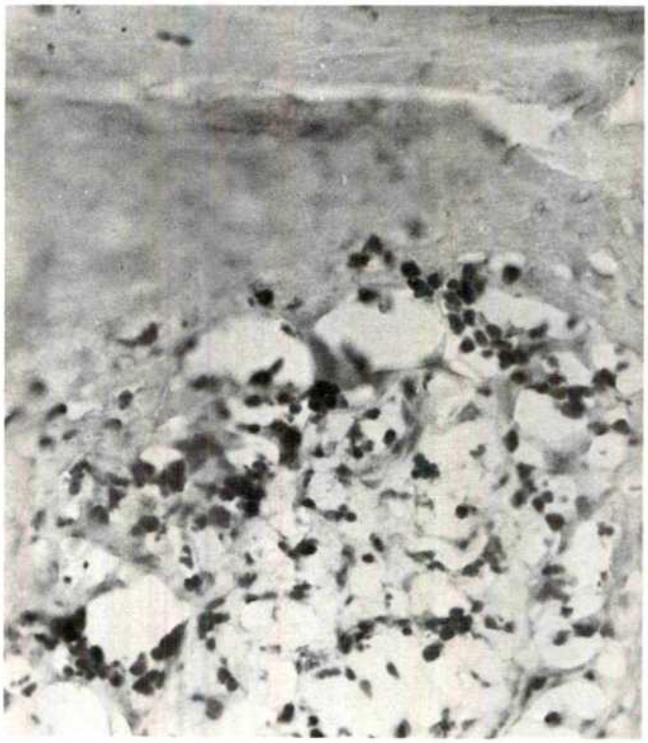

FIGURE 3. Section treated with anti-lysozyme antibodies. Positive staining identifies histiocytic cells.

DISCUSSION

This case demonstrates a clear preponderance of T lymphocytes of the cytotoxic/suppressor subset in the epidermal infiltrate of Pagetoid reticulosis. This is in direct contrast to the many reports of similar studies in mycosis fungoides where the bulk of the lymphocytic infiltrate bears the T helper surface marker (Thomas *et al.*, 1982; Mackie & Turbitt, 1982, 1983). Although we have seen a small number of suppressor/cytotoxic lymphocytes admixed with the helper lymphocytes in early mycosis fungoides, the density of the cytotoxic lymphocytes in this case is very much greater than that observed in such cases. The density of the cytotoxic suppressor cells in this case of Pagetoid reticulosis is also very much denser than that seen in benign conditions such as nickel contact dermatitis.

The second cell population present in the infiltrate in this case would appear to be histiocytic in origin in view of the staining with anti-lysozyme antibodies. This would confirm the observation by Chu & MacDonald (1980) of the presence of histiocytes in this condition. In contrast, however, to Geerts *et al.* (1982), we cannot confirm the presence of excessive numbers of Langerhans cells as the NAI 34 membrane marker revealed only a normal Langerhans cell complement.

This is the first full report in the literature of immunohistological evaluation of the lymphoid infiltrate in Pagetoid reticulosis, and it suggests that Pagetoid reticulosis is an entity that should be considered distinct from mycosis fungoides. The slow growth pattern, the extreme radiosensitivity and the isolation to one body site are also features suggesting that the condition should be considered as a separate entity.

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Syringolymphoid hyperplasia with alopecia

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SUMMARY

A 26-year-old man presented with an unusual skin lesion consisting of a hairless area on the left leg studded with reddish-brown papules, together with follicular hyperkeratosis and circumscribed anhidrosis. Skin biopsies showed dense lymphocytic infiltrates in the dermis and around hyperplastic sweat ducts and glands. Similar clinical and histological features have been described in two previous case reports. We propose the term syringolymphoid hyperplasia with alopecia for this unusual skin disease.

In this paper we report an unusual skin disease which has apparently been previously described in only two other cases (Sarkany, 1969; Steigleder & Bauermeister-Jasso, 1981).

The clinical features consist of patchy hairless areas of skin with follicular, reddish-brown papules, anhidrosis and diminished sensibility. Ulceration may occur in the affected regions. The histological features are specific for this dermatosis.

CASE REPORT

A 26-year-old man was first seen in March 1979 because of a hairless area with follicular reddish-brown papules on his left leg. The skin changes had appeared at age 24, 2 years before the consultation. During the last year, ulcerations had occurred in this area.

On physical examination, a 20 × 25 cm hairless skin lesion was noted on the left leg, characterized by a reticular erythema (Fig. 1), follicular reddish-brown papules with hyperkeratosis (Fig. 2) and several flat ulcers. This area showed diminished sensation and complete absence of sweating, even after stimulation by physical effort (Fig. 3).

Further examination, including a search for vascular, ophthalmological and pulmonary changes, showed no defects, and routine laboratory tests were normal. The patient has been followed for nearly 4 years. He is now 29 years old and in good general health.

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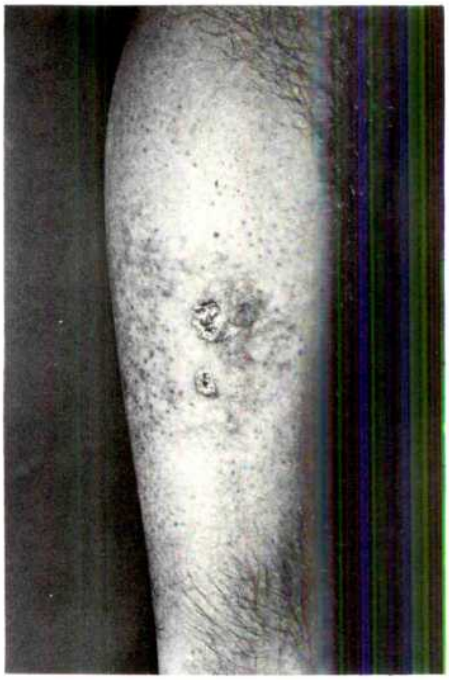

FIGURE 1. Hairless skin lesion with reticular erythema, follicular reddish-brown papules and hyperkeratosis.

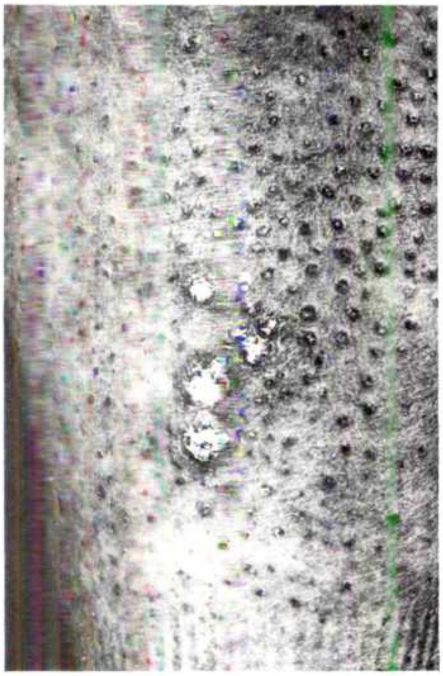

FIGURE 2. Detail of Fig. 1. Follicular papules with hyperkeratosis.

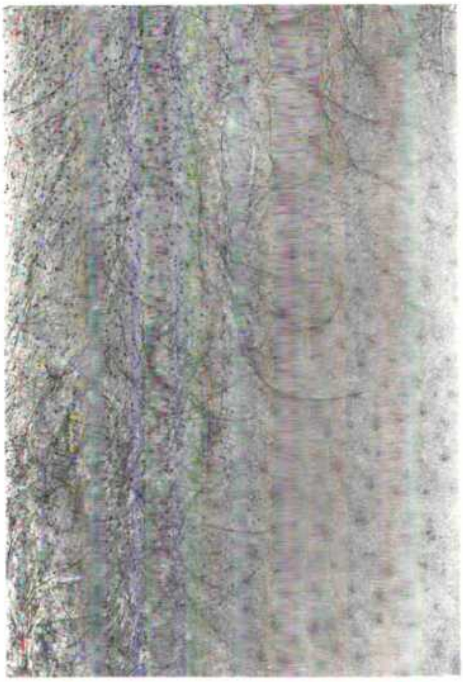

FIGURE 3. Sweat testing: Showing absence of sweating in the hairless area.

Histopathology

Six skin biopsies were taken on several different occasions. For histological examination, the paraffin-embedded sections were stained with haematoxylin and eosin, Giemsa, a combination of Alcian blue and PAS, van Gieson, toluidine blue, and Fite.

The sections obtained from the reddish-brown papules showed dense lymphohistiocytic infiltrates in the upper dermis and around the hair follicles. These infiltrates contained occasional foreign body giant cells. The mid and deeper dermis contained hyperplastic dermal ducts and sweat coils, surrounded by a dense lymphocytic infiltrate (Fig. 4). In part, the infiltrate sleeved the sweat glands and penetrated their hyperplastic walls (Fig. 5). The lumina of the dermal ducts were partly absent. These findings persisted in different biopsies over a 2-year observation period. Special staining procedures (see above) revealed no further information.

For direct immunofluorescent (DIF) examination, 4-μm thick frozen sections were incubated with FITC-conjugated goat anti-human IgG, IgA, IgM, C3 and fibrinogen antibodies. No specific staining patterns were shown by DIF examination with any of the antisera used.

For immunohistochemical analysis, acetone-fixed frozen sections were incubated with mouse monoclonal antibodies against human T-lymphocyte subsets, Langerhans cells and HLA-DR antigens (Ortho Pharmaceutical Company, Raritan, N.Y.). Positive reactions were visualized by a two-step immunoperoxidase technique using 3-aminoethylcarbazole as a substrate for peroxidase. The reactivity of these reagents was described by Poppema *et al.* (1981).

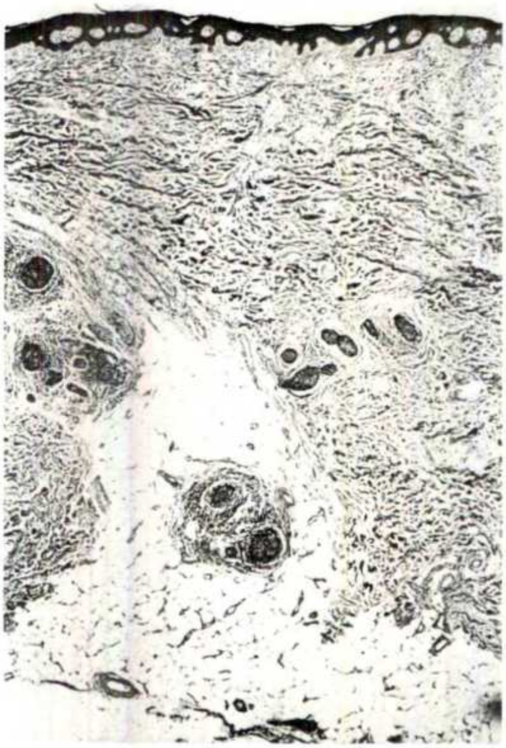

FIGURE 4. Lymphocytic infiltrate surrounding hyperplastic sweat duct and glands.

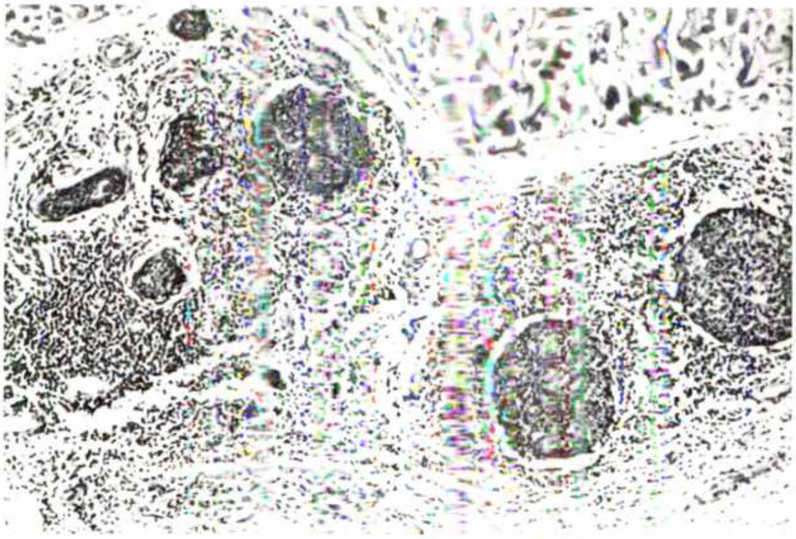

FIGURE 5. Dense lymphocytic infiltrates sleeving the sweat glands and partly penetrating their hyperplastic walls.

Immunohistochemical analysis (Table 1). The major component of the lymphocytic infiltrate consisted of activated T lymphocytes (OKT3$^+$, OKT11$^+$, OKIa1$^+$). The phenotypic markers of helper (OKT4) and suppressor/cytotoxic (OKT8) T cells were present in the same ratio as in normal peripheral blood i.e, T4$^+$:T8$^+$ approximately 2:1. The epidermal Langerhans cells (OKT6$^+$, OKIa1$^+$) were found to be increased, and OKT6$^+$ cells were present in the infiltrate and within the altered sweat ducts. The keratinocytes in some parts of the epidermis were weakly stained with HLA-DR antibody (OKIa1) and so were the cells of the hyperplastic sweat glands.

TABLE 1. Antigenic markers

	Epidermis	Mononuclear infiltrate	Altered sweat coils
OKT3, OKT11 (mature T lymphocytes)	Few cells positive	Approx. 70–80% cells positive	Few cells positive
OKT4 (helper subset)	0	Approx. 60% cells positive	0
OKT8 (cytotoxic/suppressor subset)	Few cells positive	Approx. 25% cells positive	Few cells positive
OKT6 (Langerhans cells)	High number of dendritic cells positive	Few cells positive (approx. 3%)	High number of cells positive
OKTIa1 (HLA-DR on B cells, macrophages, activated T cells)	Keratinocytes: partly positive high numbers of dendritic cells positive	Nearly all cells positive	Epithelial and dendritic cells positive

DISCUSSION

In 1969, Sarkany presented a man with patchy alopecia, anhidrosis, eccrine gland wall hypertrophy and vasculitis at the Royal Society of Medicine. The patient showed pinkish-white, bald patches on his chest, arms, forearms, hands, and scalp and painful ulcers on the heels. He also admitted to loss of sweating in these areas. The histological examination showed 'grossly hypertrophic sweat coils which were tightly sleeved by a dense lymphoreticular infiltrate'. This same patient was later reported under the diagnosis of lymphomatoid granulomatosis by MacDonald & Sarkany (1976) because the histopathological features resembled those of a patient presented by MacDonald (1975) at the Royal Society of Medicine with this diagnosis.

Six years later, Steigleder & Bauermeister-Jasso (1981) described a patient with similar features comprising a patchy alopecia, reddish-brown follicular papules and hyperkeratosis on both arms, the right thigh and the left ankle. The histological examination revealed findings identical to those observed with our own patient and the patient of Sarkany. With reference to the report of MacDonald & Sarkany (1976), they labelled their case 'lymphomatoid granulomatosis with epithelial islands'.

The patient observed by MacDonald presented X-ray findings suggestive of lymphomatoid granulomatosis. Unfortunately it was not possible to confirm this diagnosis by autopsy because of organ autolysis. The diagnosis was however supported by the subsequent publication of Holden, Wells & MacDonald (1982) who reported a case of cutaneous lymphomatoid granulomatosis with similar skin manifestations to the patient described by MacDonald (1975).

Our patient's skin lesions, as well as the skin lesions of the two patients described in the literature, differ from those described in patients with lymphomatoid granulomatosis (Liebow, Carrington & Friedmann, 1972; Kay *et al.*, 1974; Bender *et al.*, 1978; Katzenstein, Carrington & Liebow, 1979; James, Odom & Katzenstein, 1981; Holden *et al.*, 1982) (Table 2). Angionecrotic

TABLE 2. Comparison of lymphomatoid granulomatosis and syringolymphoid hyperplasia with alopecia

	Lymphomatoid granulomatosis	Syringolymphoid hyperplasia with alopecia
General health	Poor	Good
X-ray findings	Multiple rounded pulmonary infiltrates	Normal lung, or non-specific infiltrates
Skin	*Transient* Macular erythema, maculopapular eruption, ulcer, subcutaneous nodules	*Persistent* Reticular erythema, brown-red follicular papules, ulcer, focal alopecia, focal anhidrosis
Histopathology	Angionecrotic destructive vasculitis, polymorphic infiltrate with lymphocytes, histiocytes, plasma cells, frequent mitoses and atypical cells, destruction of appendages	Occasional vasculitis, subepidermal dense lympho-histiocytic infiltrate, sweat gland hyperplasia surrounded by lympho-histiocytic infiltrate
Peripheral neuropathy	Paraesthesia of the lower extremities (in 7%)	Temporary focal diminished sensibility
Prognosis	Poor	Apparently good

TABLE 3. Comparison of the 3 cases of syringolymphoid hyperplasia with alopecia so far reported

	Diagnosis	Localization	Clinical features	Histological features
Sarkany, 1969	Patchy alopecia, anhidrosis, eccrine gland wall hypertrophy and vasculitis	Chest, arms, forearms, hands, scalp	Pinkish-white bald patches with prominent follicular markings, ulceration, anhidrosis	'Hypertrophic sweat coils surrounded by a dense lymphoreticular infiltrate'
Steigleder & Bauermeister-Jasso, 1981	Lymphomatoid granulomatosis with 'epithelial islands'	Arms, legs	Patchy alopecia with reddish-brown papules, follicular hyperkeratosis, erythema	Hyperplastic eccrine glands surrounded by a dense 'lymphadenoid' infiltrate
Vakilzadeh & Bröcker, 1983	Syringolymphoid hyperplasia with alopecia	Left leg	Circumscribed alopecia with reddish-brown papules, follicular hyperkeratosis, reticular erythema ulceration, anhidrosis	Hyperplastic eccrine glands surrounded by a dense lymphocytic infiltrate

destructive vasculitis, which is the main feature of lymphomatoid granulomatosis, could not be demonstrated by histology.

In Table 3, the clinical and histological features of our case are compared with those of the two previous case reports. In our opinion, these patients suffered from a distinct dermatosis which seems to be independent from and not related to lymphomatoid granulomatosis.

The immunohistological investigations (Table 1) revealed infiltrates of activated, mature T lymphocytes. The altered sweat coils within the mononuclear infiltrate contained many Langerhans cells. Moreover, their epithelium appeared to be HLA-DR positive.

As long as the aetiology and pathogenesis of this skin disease are unknown, it seems reasonable to designate it according to its main clinical and histological features, namely alopecia and hyperplasia of sweat glands surrounded by dense lymphoid infiltrates. We therefore propose for this disease the term 'syringolymphoid hyperplasia with alopecia'.

ACKNOWLEDGMENT

We thank Dr P.J.Frosch for performing the sweat testing.

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Atypical eruptive histiocytosis—a marker of underlying malignancy?

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SUMMARY

An eruptive histiocytosis was the presenting feature in a patient who developed acute myelomonocytic leukaemia. The differential diagnosis of the skin lesions is discussed and in the light of a recent similar report we propose that the two disorders are associated.

Proliferation of cells of the histiocyte/monocyte series may be found in a variety of clinically distinct syndromes, ranging from xanthogranuloma to eruptive histiocytomata, reticulohistiocytoma and histiocytosis X. These proliferative processes behave in a biologically non-malignant manner despite the locally aggressive nature of histiocytosis X and are not associated with underlying malignant disease.

Recently O'Donnell *et al.* (1982) reported a case of acute myelomonocytic leukaemia presenting with a xanthomatous skin eruption. We report a similar case and discuss the classification of this 'eruptive histiocytosis' and its relationship to monocytic leukaemia.

CASE REPORT

A 66-year-old woman presented with ten firm skin nodules, ranging from 4 mm to 2 cm in diameter, which were widely distributed over the trunk and limbs. In colour they varied from pink to a bluish red, several of the lesions showing a superimposed yellow hue.

The patient's general health was good; there was no history of arthropathy and she was taking no medication. Apart from the cutaneous lesions general examination was unremarkable.

Several lesions were biopsied, ranging from an early nodule which had been present for about 1 month, to larger, older lesions of several months' duration. All lesions showed a proliferation of eosinophilic histiocytes in the superficial dermis; the earliest lesion also contained a sparse lymphocytic infiltrate. In the larger lesions many of the histiocytes contained lipid as demonstrated by Congo Red and Sudan black stains and in the superficial part of the lesion there

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were numerous foreign body giant cells. The deeper parts of the nodule showed a dense fibroblastic proliferation in a storiform pattern with a patchy infiltrate of histiocytes and lymphocytes. No eosinophils were seen in any of the leasions and Perls' stains showed no haemosiderin deposition. The histiocytic cells in all lesions showed little nuclear pleomorphism and very few mitoses. It was concluded that the lesions represented a benign histiocytosis with variable lipid and fibrous components.

Further investigations showed a neutropenia (white cell count 2·5 × 10^9/l, 33% neutrophils, 57% lymphocytes, 10% monocytes). The haemoglobin, red cell indices and platelet counts were normal. The sedimentation rate, biochemical screen, immunoglobulins, protein electrophoresis, serum lipids and chest X-ray were also normal. Anti-nuclear factor was negative. Given the patient's general well being, a bone marrow examination was not performed at that stage.

Over the following 16 months approximately fifty new lesions appeared, sometimes following minor trauma; the nasal mucosa was also affected. There were no ocular lesions. Throughout this period the patient remained in good health and there was no alteration in the haematological indices.

Eighteen months after her initial presentation she complained of lethargy, pallor and bruising and some of the lesions became purpuric, whilst others showed signs of resolution. Investigations were as follows: haemoglobin 9·5 g/dl, white cell count 18·7 × 10^9/l, 36% neutrophils, 8% lymphocytes, 56% atypical cells of monocytoid appearance, platelets 14 × 10^9/l. A bone marrow aspirate showed gross infiltration with pleomorphic blast cells, and antigen marker studies showed this to be an acute leukaemia of monoblastic/histiocytic origin.

Despite intensive therapy the patient died 2 days later. A post mortem was not performed.

DISCUSSION

The classification of cutaneous non-X histiocytosis is based upon a combination of clinical and histological features. The topic has been extensively reviewed by Winkelmann (1981) who postulates a common stem cell precursor to the blood monocyte, the macrophage-histiocyte and the Langerhans cell. The benign non-X histiocytoses represent focal benign histioxanthomatoses that often undergo spontaneous resolution and comprise the following entities: eruptive histiocytoma, juvenile xanthogranuloma, papular xanthoma, xanthoma disseminatum, necrobiotic xanthogranuloma and reticulohistiocytoma cutis. The precise differentiation of these entities is not always clear cut; the earliest lesion in many cases is a pure proliferation of histiocytic cells which lack Langerhans granules. Later lesions show varying combinations of histiocytes, lipid-laden histiocytes, multinucleate giant cells, haemosiderin deposition and fibrous proliferation.

Our case shows the proliferation of histiocytic cells and several clinical features in common with eruptive histiocytoma but in addition has numerous lipid-laden histiocytes and multinucleate giant cells which are features of papular xanthoma (adult xanthogranuloma). However, the degree of fibrous proliferation and the lack of eosinophils are against this diagnosis histologically. Taunton, Yeshurun & Jarratt (1978) described a case under the heading of progressive nodular histiocytoma (PNH); a further case was reported by Burgdorf *et al.* (1981). This comprises a syndrome of nodular histioxanthomatous proliferation with an admixture of giant cells and haemosiderin deposition. Unlike the other entities described, the cases of PNH have shown no tendency to spontaneous resolution. This case bears a close clinical and histological resemblance to PNH; however, Winkelmann (1981) has thrown doubt on the existence of PNH as a distinct entity. It is perhaps not surprising that where several syndromes have a common cellular origin, a degree of histological and clinical variability may also occur and the term atypical eruptive histiocytosis serves to stress the common origin of these benign histiocytic proliferations which cannot be clearly categorized.

The possibility that an atypical eruptive histiocytosis may be a marker for an underlying neoplasia is suggested by the simultaneous proliferation of the bone marrow monocyte and the cutaneous histiocyte in our case. The report of O'Donnell *et al.* (1982) of an 'unusual xanthomatous lesion' as the presenting feature of an acute myelomonocytic leukaemia lends support to this being more than a chance association. Furthermore, another histiocytosis, generalized plane xanthoma, is associated with either leukaemia or multiple myeloma in a high proportion of cases (Lynch & Winkelmann, 1966). The mechanism leading to stimulation of two related though independent cell lines remains obscure.

ACKNOWLEDGMENT

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Centrifugal lipodystrophy

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SUMMARY

We describe a case of centrifugal lipodystrophy occurring in a young English girl, and compare the clinical and histopathological features with previous reports from Japan.

Imamura, Yamada & Ikeda (1971) described five Japanese children with lipodystrophia centrifugalis abdominalis infantilis. We now describe a young English girl with this condition and we believe this is the first case to be reported in Europe.

CASE REPORT

A 3-year-old girl presented with a cutaneous depression over the lumbar region. This lesion, noted to be present at birth, had always been covered by normal-looking skin and was gradually enlarging in a centrifugal distribution. There were no specific symptoms apart from occasional discomfort and bruising following trauma to this area.

She was the product of a full term normal pregnancy and delivery, her parents being unrelated English Caucasians.

On examination she was an intelligent girl who had achieved the normal milestones for her age. General examination was entirely normal. There was a large circular depressed area over the left lumbar region extending across the thoraco-lumbar spine to the right side and to the anterior abdominal wall on the left (Fig. 1). A distinctive slightly erythematous raised border surrounded the entire lesion. The underlying vasculature could be clearly seen giving rise to a bluish tinge. Sensation was normal and there was no evidence of scarring. Regional lymphadenopathy was not noted.

INVESTIGATIONS

An elliptical biopsy was taken from the lower margin of this lesion. The most striking feature was the abrupt reduction in the amount of adipose tissue from relative abundance at the periphery of the lesion to a thin and narrowing strip of fat cells sandwiched between the dermis and underlying fascial tissue (Fig. 2). While the epidermis appeared normal the dermis had a

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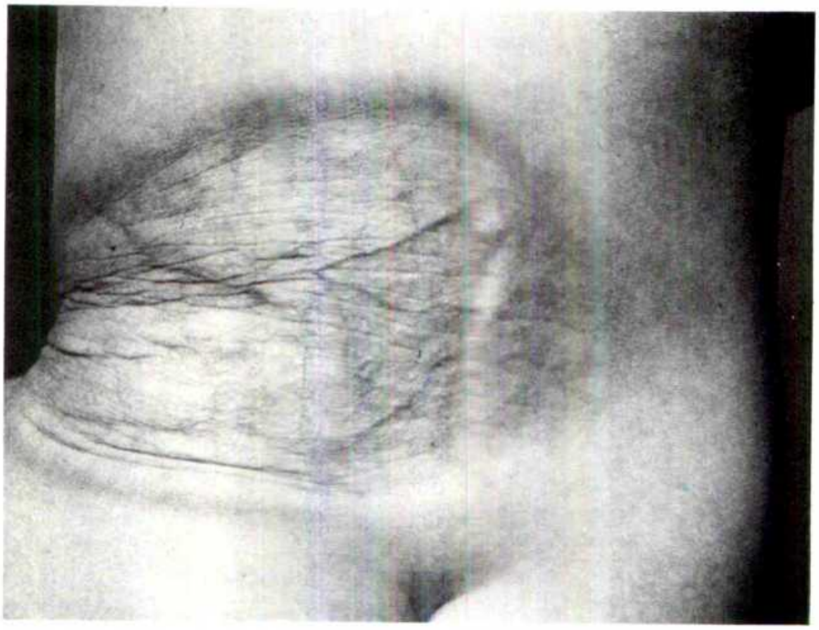

FIGURE 1. Centrifugal lipodystrophy extending across thoraco-lumbar spine to the right and anterior abdominal wall on the left. The raised erythematous margin and underlying vasculature are clearly seen.

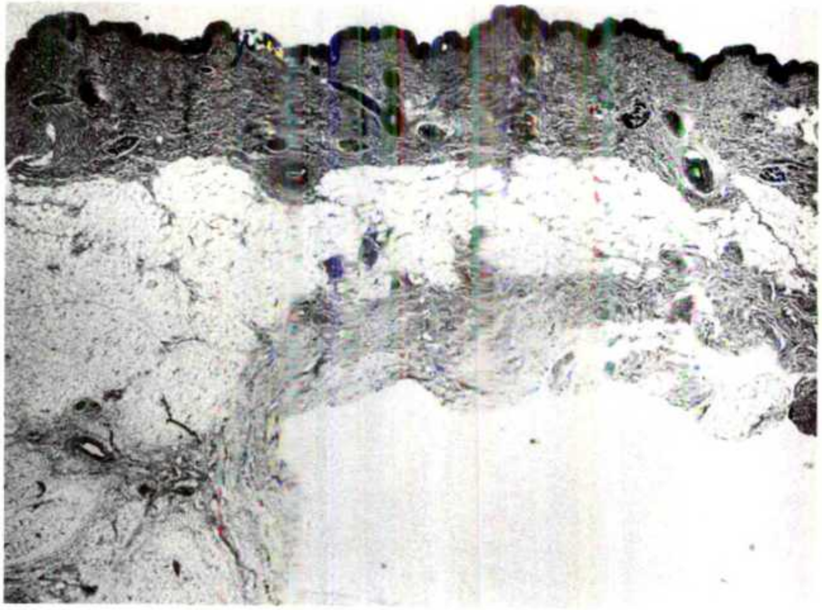

FIGURE 2. Abrupt thinning of subcutaneous adipose tissue at margin of lesion leaving a thin strip of fat cells between the dermis above and fascia below.

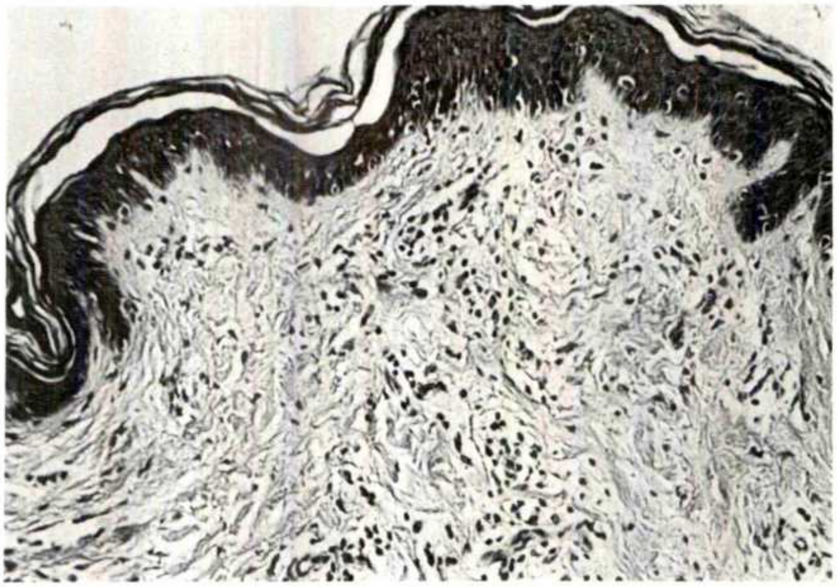

FIGURE 3. Mixed mononuclear cell infiltrate in dermis.

rather non-specific and mild mixed inflammatory infiltrate with no obvious abnormality of collagen or elastic tissue (Fig. 3). The skin appendages were well preserved. There was no associated inflammatory infiltrate in the fat and in particular there was no evidence of fat necrosis. The underlying fascia appeared normal.

Radiology of the spine and pelvis was unremarkable.

Since our patient's first presentation she has unfortunately developed acute lymphoblastic leukaemia and is currently receiving cytotoxic therapy.

DISCUSSION

Five individuals with this rare condition were reported by Imamura *et al.* (1971). Since that time at least eighty-three patients have been diagnosed in Japan (Imamura, 1981). The geographical concentration of this disease is highlighted by the fact that only one other case has been reported elsewhere, namely in Singapore (Imamura, 1981; Giam, Rajan & Ong Beng Hock, 1982). The case reported by Cairns (1976) fails to fulfil the distinctive clinical and histological criteria now required for this condition (Imamura & Yamada, 1977).

The age of onset ranges from 1 month to 9 years, 50% developing lesions by 4 years and 80% by 5 years of age. Although the aetiology is unknown, localized congenital or acquired abnormalities such as inguinal herniae, lymphadenopathy and bruising have been noted to occur in this condition and may have induced the dystrophic process.

There is a predilection for the groins and surrounding areas in 80% of cases, whereas the axillae and upper chest are involved in only 20%. Presentation follows gradual awareness on the part of the parents of a depression of the skin with an associated bluish tinge. Examination reveals subcutaneous atrophy, well demarcated by a raised, slightly scaly and erythematous border. The subcutaneous blood vessels can be clearly seen to traverse this area. Regional lymphadenopathy is said to occur in about 70% of cases.

General examination is normal, but in some cases laboratory studies have shown abnormalities of white cell count, in particular lymphocytosis and occasionally leukopenia. This is of particular interest in view of the recent development of leukaemia in our patient. Cases have occurred in dizygotic twins, and one patient had consanguineous parents (Mizoguchi & Nanko, 1982), suggesting that heredity may play a role in the pathogenesis of this disease.

Histological examination reveals no epidermal change. The characteristic minimal dermal infiltrate is probably non-specific. Epidermal appendages are well preserved and collagen and elastin are usually normal. Subcutaneous fat is usually absent at the centre of the lesion and markedly thinned at the periphery. A moderate amount of inflammatory infiltrate consisting of lymphocytes, histiocytes and plasma cells has been noted in most cases, although this did not apply to our case.

The areas of subcutaneous depression gradually enlarge centrifugally from the original sites over a period of years. Exacerbations have occurred in relation to acute bouts of fever. Growth of these abnormal areas ceased spontaneously in 90% of the cases by the age of 9 years and in at least one-third of these cases the lesions subsequently become inconspicuous.

In the short term, treatment has been generally disappointing, but some improvement has been reported with corticosteroids (orally or topically) in 71% of cases (Imamura, 1981).

A number of similarities between centrifugal lipodystrophy and progressive lipodystrophy have been noted. The distinction between these two diseases is important because up to 90% of patients with progressive lipodystrophy can develop a membranous glomerulonephritis and associated hypocomplementaemia. A high proportion of these will die from renal failure within 10 to 25 years of the original onset of their disease (Cunliffe & Bleehen, 1979; Eisinger, Shortland & Moorhead, 1972; Peters *et al.*, 1973; Poley & Stickler, 1963).

Centrifugal lipodystrophy is a discrete entity characterized by its distinct clinical and histological findings. To our knowledge this is the first Western case reported and only the second case to be reported outside Japan. Furthermore it is most unusual for this to occur as a congenital abnormality. It now appears that the original name, lipodystrophia centrifugalis abdominalis infantilis, is perhaps inappropriate since the condition may affect areas other than the abdomen and occur outside infancy.

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Society Proceedings

Proceedings of the 228th Meeting of the Netherlands Society of Dermatology and Venereology, Amsterdam, 7 May 1983

PRESIDENT: PROFESSOR DR E. VAN DIJK
HOST: PROFESSOR DR E. VAN DIJK

SUMMARIES OF PAPERS

Acrokeratosis paraneoplastica (Bazex syndrome)

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This condition was first described by Bazex and co-workers in 1965 as a skin marker of malignancy.

The malignancy is a squamous cell carcinoma in the laryngo-pharyngeal region. The dermatological features are erythematous scaly lesions on the extremities, ears and face combined with dystrophic nails of fingers and toes with onycholysis and subungual hyperkeratosis. Treatment of the carcinoma usually leads to clearing of the skin lesions.

Most published cases are French with a few cases from Spain (Cabre *et al.*, 1975) and the U.S.A. (Braverman, 1981). The condition affects males exclusively, mostly over the age of 40 (Bazex & Griffiths, 1980). There is one exception: a woman aged 53 presented by Civatte in 1980. She had a squamous cell carcinoma in the upper right bronchus (personal communication, Professor J.Civatte). We report a female patient; the first case reported in Holland and probably the second female patient published with this condition.

A woman, aged 57, in the past year had developed psoriasiform scaly lesions on the hands and feet, especially on the fingers and toes. There was violaceous erythema with hyperkeratosis which had a typical honeycomb appearance on the fingers and toes (Fig. 1). Later on similar lesions developed on the earlobes and nose. There was a scaly violaceous erythema on the wrists, elbows and knees with slight infiltration of the skin. The nails of fingers and toes were dystrophic with onycholysis and subungual hyperkeratosis.

A biopsy from the back of the right hand showed hyperkeratosis with a few very small parakeratotic foci and mild acanthosis. In the papillary layer many capillaries were present. There was a mainly perivascular lymphohistiocytic infiltrate in the superficial and deeper part of the dermis. Some of the dermal papillae were elongated with a thin suprapapillary plate which had a psoriasiform appearance. However the histopathological changes were not specific for psoriasis or lupus erythematosus, diagnoses which were suggested by the clinical picture. At the same time an undifferentiated squamous cell carcinoma of the upper right bronchus was diagnosed. It was inoperable as it penetrated the right pulmonary vein. Ten months after the first consultation the patient died at home. An autopsy was not performed.

Although acrokeratosis paraneoplastica is a rare condition it is important to recognize it, for in some cases it will be possible to make the diagnosis before the malignant tumour is inoperable.

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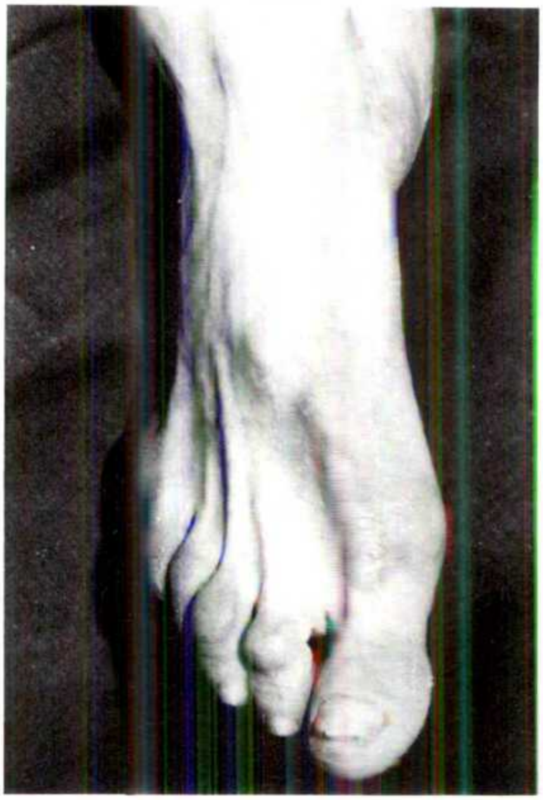

FIGURE 1. Acrokeratosis paraneoplastica, showing erythema and hyperkeratosis.

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Immunological investigations in patients with drug-induced skin eruptions

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In the past 10 years, 775 patients with a clear history of skin eruptions due to drugs have been examined by means of *in vivo* tests. Epicutaneous test reactions appeared to be positive under certain conditions:

(1) After external sensitization to a topically applied drug. In these cases application of chemically related drugs, which when used systemically may sometimes cause generalized eczematous eruptions, may lead to prevention of these reactions.

(2) In certain groups of drugs: pyrazolone derivates, psychotropic drugs (phenytoin, carbamazepine), chemotherapeutic drugs (sulphonamides, nitrofurantoin) and cardiovascular drugs (β-blockers).

(3) In penicillins, especially the aminopenicillins.

In groups 1 and 2 the number of patients with one or more positive patch test reactions was about 15%. In 34 out of 130 patients who had shown eruptions after therapy with penicillins, positive patch test reactions were observed. Most patients with a so-called 'toxic rash' (29) had positive reactions to ampicillin and amoxycillin. The group allergic pattern showed a complete cross-sensitization for ampicillin and amoxycillin. Fourteen patients showed group allergic reactions for the amino-penicillins and pheneticillin. Cross-sensitization between the penicillins and cephalosporins was observed only in 9% of the patients of this group. In 38 out of 130 patients with negative patch test reactions, positive immediate type reactions were seen by intracutaneous testing. The testing procedure was performed by using PPL-6 (Cilligen®) and benzylpenicillin. Cilligen® contains the major haptenic determinant (the penicilloyl group) which is coupled to polylysine. In sixteen out of thirty-eight patients with positive intracutaneous reactions, the clinical reactions indicating an immediate type reaction (anaphylactic shock, urticaria) had been seen after benzylpenicillin had been injected. However, positive intracutaneous reactions were also observed in ten patients with a history of the so-called 'toxic' ampicillin or amoxycillin rash. These observations demonstrate the possible danger of anaphylactic reactions in patients with an ampicillin or amoxycillin rash when these preparations or any other penicillin are prescribed again.

It may be concluded that, in 72 out of 130 patients (about 55%) with a history of allergy to penicillin, this allergy was established by *in vivo* tests. From the total group of 775 patients with a history of drug eruptions about 30% showed positive epicutaneous or intracutaneous tests. Half of these patients appeared to be allergic to penicillin.

Subacute cutaneous lupus erythematosus

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Recently, several authors have focused attention of a distinct subset of lupus erythematosus now generally referred to as subacute cutaneous LE (SCLE). This form of LE is clinically well defined as a recurring, superficial, non-scarring type of LE, consisting of a papulosquamous or annular-polycyclic eruption with a characteristic distribution over face, neck, upper part of back and chest, shoulders, extensor surfaces of arms, hands and fingers (photodistribution). In this paper the results of extensive studies in twenty-three patients are discussed and compared with those obtained by Sontheimer, Thomas & Gilliam (1979) in their group of twenty-seven patients.

The results of laboratory investigations are given in Table 1. Histopathological findings may vary considerably between different patients and in the same patient during the course of the disease. However, some features are constantly present to a greater or lesser extent (Table 2).

Immunofluorescence studies reveal abnormalities in about 50% of the patients. Partly these findings are quite different from the usual findings in LE; they will be published in due course. For the treatment of SCLE there are several possibilities. Sometimes topically applied corticosteroids are sufficient; if not, hydroxychloroquine is administered by mouth, with excellent results. Ultimately thalidomide can be prescribed.

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TABLE 1. Laboratory findings

	Own series	Sontheimer *et al.*
ESR increase	10/23 = 43·48%	16/27 = 59%
WBC decrease	11/23 = 47·83%	5/27 = 19%
Thrombocyte decrease	8/23 = 34·78%	
γ-glob. increase	9/23 = 39·13%	8/27 = 30%
LFT abnormal	4/23 = 17·39%	
IgA increase	8/23 = 34·78%	
IgG increase	6/23 = 26·09%	
ANA	8/23 = 34·78%	17/27 = 63%
Anti-dsDNA	2/23 = 8·70%	10/27 = 37%
Anti-ENA	6/23 = 26·09%	
(SM)	3/6 = 50%	
(RNP)	3/6 = 50%	
Cryoglobulin	3/23 = 13·04%	
SIC	3/23 = 13·04%	
Rheumatoid factors	5/23 = 21·74%	5/27 = 19%
FPBTS	1/23 = 4·35%	2/27 = 7%
Complement decrease	9/23 = 39·13%	6/27 = 22%

LFT: liver function tests; SIC: soluble immune complexes; FPBTS: false positive blood tests for syphilis; ENA: extractable nuclear antigen; SM: non-nucleic acid antigen; RNP: nucleoprotein antigen

TABLE 2. Histopathology

Epidermis
  Orthokeratosis ± focal parakeratosis
  Atrophy
  Dyskeratosis
  Liquefaction degeneration
  Thickened basement membrane
Dermis
  Moderately dense lymphocytic infiltration around vessels (and adnexa)

The diagnostic significance of the immunofluorescence method in comparison with the histopathology in discoid lupus erythematosus (DLE) and lichen planus (LP)

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Positive immunofluorescence (IF) patterns in LP and DLE are usually highly characteristic. However the IF diagnosis is not always easy. Factors influencing the positivity and significance of the IF pattern are duration and site of the lesions, the possible overlap with other diseases (Jablonska, Chorzelski & Beutner,

1979) and with normal skin (Nieboer, 1981), and a certain similarity between the pictures of LP and DLE (Abell *et al.*, 1975; Baart de la Faille-Kuyper & Baart de la Faille, 1974). In a study consisting of three parts, the significance of the IF method was investigated. In the first part the incidence and morphological patterns of the Ig, complement factors C1, C4, C3, C5, properdin, fibrinogen and albumin were estimated in 92 DLE and 114 LP IF-positive biopsies. It appeared that fibrinogen, IgM, C3 and cytoid bodies are frequently present in both diseases.

Diagnostic confusion is possible if IgG is absent in the case of DLE or, on the other hand, if it is present in LP, which seldom occurs. In the second part the incidence of false-positive and false-negative conclusions was investigated in DLE (sixty-eight biopsies), DLE-like diseases (fifty-nine biopsies), LP (fifty-seven biopsies) and LP-like diseases (thirty biopsies). Both IF-positive and negative biopsies from patients with clinically well-established diagnoses were included. The most important findings were the relatively high rate of false-negative (19%) and inconclusive (16%) IF diagnoses in DLE. On the other hand, in the DLE-like group, false-positive results were obtained in only 2% and inconclusive results in 6%. For LP and the LP-like group these figures were respectively 6, 7, 0 and 10. In the third part these results were compared with those from the histopathology (PA). Although the IF results were better than the PA results, these differences appeared to be not significant. The results from the combined IF and PA investigations in simultaneously taken biopsies were statistically significantly better than those from the PA but not better than those from the IF.

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Immunoenzyme histochemistry and dermatoses

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Immunoperoxidase methods were introduced in 1966, and have been applied in the field of dermatology from the early 1970s onward. The methods appeared to be as reliable as common immunofluorescent methods (Boorsma, Nieboer & Kalsbeek, 1975), but the field of application is broader. Plasmacellular proliferations can now be studied in paraffin sections for monoclonality and immuno-electron microscopy of skin diseases has become possible (Nieboer *et al.*, 1980).

By using other enzymes as markers the immunoenzyme methods extended. The most prominent alternative enzyme is alkaline phosphatase, which can be successfully applied in cases where endogeneous peroxidase activities cause unacceptable interference. Large amounts of granulocytes cause these situations. A wide variety of histochemical substrates for this alkaline phosphatase is available, thus almost every wanted colour can be obtained.

Another stable marker enzyme is glucose oxidase. Preliminary experiments have shown that this enzyme can be used as a marker in immunohistochemical studies. However, at present there is only one suitable histochemical substrate. The next logical step is immunoenzyme double staining (Boorsma, 1983).

Recently this double staining was developed by coupling different enzymes to different primary antibodies. The conjugates obtained were applied on sections as a 'cocktail' for demonstrating two different antigens in one tissue section. This method is very suitable for the application of monoclonal antibodies in double staining. Plasma cells containing different immunoglobulins could be detected simultaneously in one tissue section. In dermal infiltrates B- and T-cell areas could be clearly distinguished.

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CASE REPORTS

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Pemphigus (?) presenting as erythema annulare centrifugum

M.J.M.POST

History. Male, aged 27 years. Since 1975 recurrent itchy annular lesions were present on trunk, arms and thighs that tended to grow eccentrically. He never used any drugs and had always been healthy.

Examination. On the trunk, arms and inner thighs ten palm-sized annular lesions with a red and raised border with rubber-like induration were present. Furthermore there were some erythematous round elevated solid patches about 1 cm in diameter.

Histology. The epidermis showed slight parakeratosis and acanthosis as well as focal spongiosis and exocytosis. There was no acantholysis. The dermis showed prominent coat-sleeve-like perivascular mononuclear infiltrates.

Immunofluorescence. Dark IF: involved and uninvolved skin showed intercellular fluorescence of the epidermis with anti-IgG, C3c and C3d; positive basement membrane with anti-C3d. Indirect IF: circulating IgG antibodies directed against cell borders of human mucosa-epithelium, in maximal titres of 1:128.

Laboratory investigation. Normal.

Course and treatment. In spite of local application of Dermovate* ointment followed by oral administration of dapsone during 3 months, new lesions developed. From May 1981 onward the lesions gradually disappeared spontaneously.

Comment. We consider this case as a remarkable one, because of the immunofluorescent characterisitics of pemphigus together with the clinical and histological picture of erythema annulare centrifugum. Although skin lesions have been absent for $1\frac{1}{2}$ years, we recently demonstrated pemphigus-like antibodies.

Epidermolysis bullosa acquisita with circulating antibodies

M.J.M.POST

History. Female, aged 53 years. Since autumn 1979 there has been blistering of arms, legs and oral mucosa following minor trauma. No intestinal symptoms. From 1964 till 1968 the patient was treated for Felty's syndrome with prednisone.

Examination. On the distal parts of the extremities about fifteen nummular white atrophic areas were seen. Also a number of erosions and small blisters were present on the toes. On the elbows and right third toe erythema and milia could be seen.

Histology. Subepidermal blister with sparse perivascular mononuclear infiltration in the dermis.

Immunofluorescence. Direct IF of both normal and peribullous skin showed a strong linear fluorescence of the basement membrane with anti-IgG, C3c and C3d and less so with anti-IgM. Indirect IF showed homologous anti-basement membrane antibodies of the IgG type (titre 1 : 16). Recently the results of this investigation were negative.

Immunoelectron microscopy. IgG deposition at the dermal side of the basement membrane.

Laboratory investigation. Rose test 1 :32, RA test positive, ANA positive, anti-DNA negative, LE-cell test weakly positive, gradually diminishing leucopenia.

Course and treatment. Phenytoin was given for 11 months and stopped, because the patient refused to take this medication any further. Meanwhile oral lesions disappeared completely, and blistering of the skin gradually diminished.

Comment. No associated disease was found in this patient with evident epidermolysis bullosa acquisita. The presence of circulating antibodies, directed against the basement membrane is remarkable. It is unknown whether these antibodies are identical with antibodies seen in pemphigoid. Clinical improvement in this disease is rare and probably occurred spontaneously in this case.

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Pigmentation secondary to minocycline therapy

A.M.Zijdenbos and K.J.Balmus

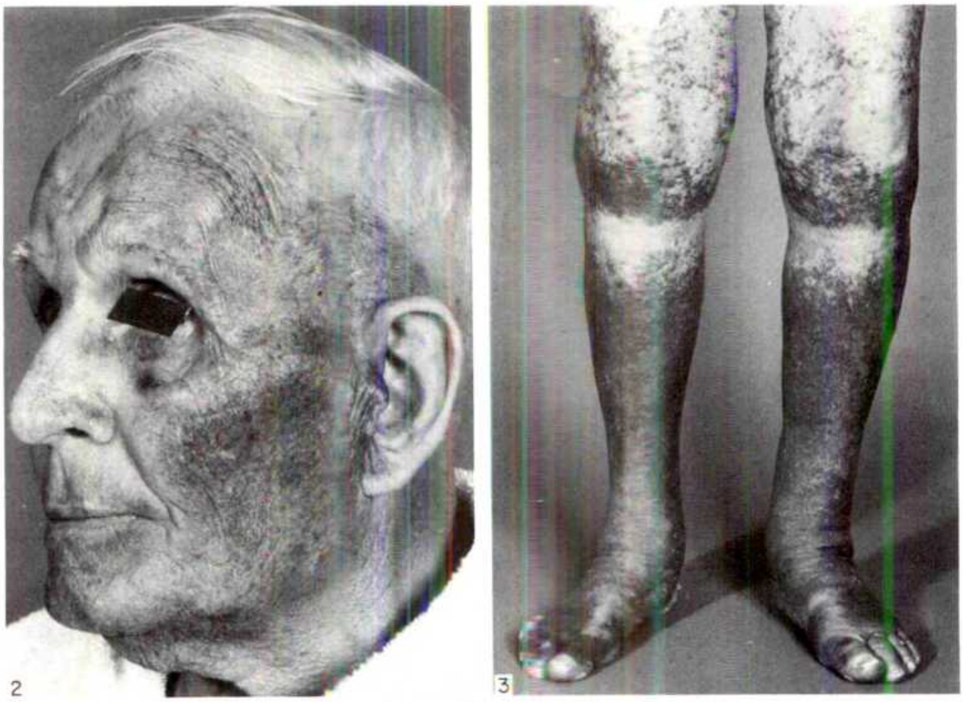
History. Male, aged 83 years. For several years this patient has shown an increasing cutaneous discoloration. There has been generalized pruritus for 1 year. He has been taking minocycline 200 mg daily for nine years for chronic pulmonary disease. There is no prior history of other drugs known to be associated with pigmentation.

Examination. Face and neck showed diffuse dark blue discoloration (Fig. 2). On the back a reticulated brown-blue hue was seen. Slightly elevated blue-black discoloration was present on feet and lower legs up to the knees, merging into a zone of erythema with multitudinous blue-black papules (Fig. 3). The hands showed erythema of skin and darkening of finger-nails, whereas the sclerae showed spotted blue discoloration.

Histology. A biopsy from a blue-black papule showed many histiocytes with brown-black pigment granules. Prussian blue and Schmorl techniques showed staining properties of melanin and iron. In addition unstained granules were found by both techniques. A biopsy from the erythema showed similar, but less pronounced findings.

*Electron microscopy.*A blue-black papule showed an increased number of melanosomes in lower epidermal keratocytes and melanocytes. Most melanocytes appear to be very active cells with melanosomes in various stages of development. Most pigment is found in both papillary and reticular dermis, mainly in phagocytic vacuoles or as more electron-dense non-membrane-bound pigment throughout the cytoplasm of histiocytes, but also in pericytes and occasionally free within the dermis.

Course and treatment. Clearing of erythema followed discontinuation of minocycline therapy. At necropsy (patient died of cardiac failure) the major findings were diffusely darkened areas of aorta, endocardium and ribs. Thyroid and prostate were black on gross examination.



FIGURES 2 and 3. Pigmentation due to minocycline therapy.

Comment. Minocycline is known to cause discoloration of the skin, thyroid, bones, teeth and finger-nails. The nature of the pigments involved and the mechanism of deposition are not clear. To our knowledge this is the first described case with pigmentation of the aorta, endocardium and prostate.

Multiple endocrine neoplasia type 2B

TH.M.STARINK

History. Female, aged 50 years. The lesions started in childhood. At the age of 25 and again at 45 the patient was treated for medullary thyroid carcinoma. The patient's mother also showed mucosal lesions.

Examination. Twenty pale yellow 1 cm nodules were present on the upper lip, the commissures of the lips, the tongue and eyelids (Fig. 4). Also mild diffuse swelling of the upper lip was present. No Marfanoid habitus was present.

Ophthalmological examination showed hypertrophic corneal nerves.

General examination and laboratory investigations. No phaeochromocytoma or hyperplasia of the adrenal medulla.

Histopathology. A biopsy from a lesion of the upper lip was typical of a neuroma.

Comment. This patient shows the typical lesions of the rare multiple endocrine neoplasia type 2B. Multiple mucosal neuromas are important marker of internal malignancy.

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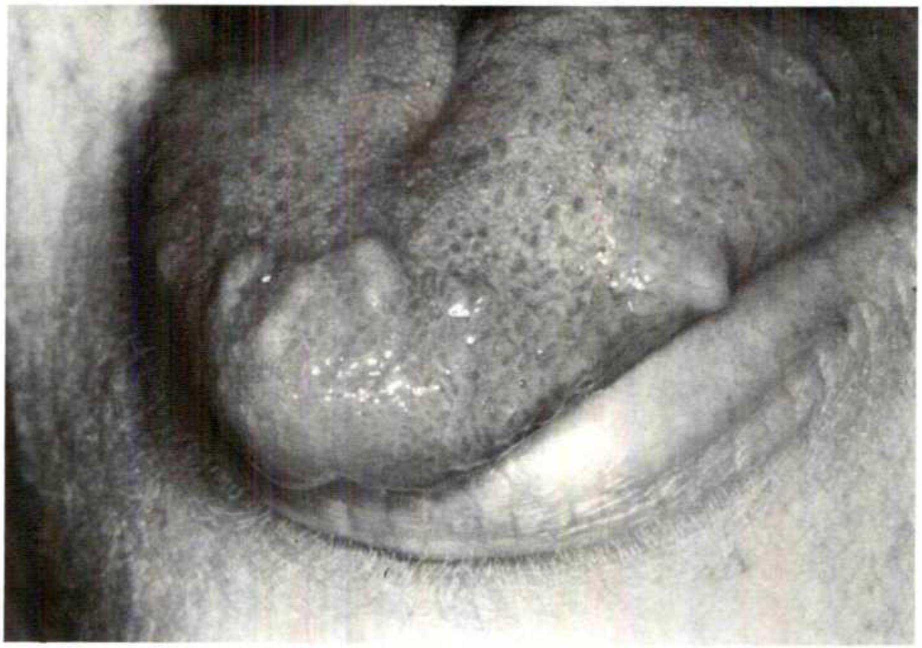

FIGURE 4. Multiple mucosal neuromas on the tongue.

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Multiple agminate juvenile melanoma in a naevus-spilus-like hyperpigmented area

M.J. WOERDEMAN

History. Female aged 7 years. Since birth a hyperpigmented area has gradually developed. During the last 5 years, reddish-brown papules have appeared. The first and largest lesion has somewhat diminished in size during the last year.

Examination. On the right cheek and temporal region there is a light-brown hyperpigmented area with some dark brown maculae and about ten reddish-brown papules varying in size from 3 to 9 mm in diameter (Fig. 5).

Histology. A biopsy from a papule showed a compound naevus consisting of large spindle cells and epithelioid cells.

Electron microscopy. Biopsies from a papule and from a dark brown macule both showed a compound naevus. In both biopsies there were giant melanosome complexes in melanocytes, naevus cells and keratocytes.

Therapy. One lesion has been treated with cryotherapy with good result. Because this treatment was painful and because of the possibility of spontaneous recovery no further treatment was performed.

Comment. Agminate juvenile melanomas are rare (Krakowski *et al.*, 1981) and especially if they occur in a hyperpigmented area (café au lait spot) or in a naevus-spilus-like hyperpigmented area. Cases like our own have been published by Duperrat & Goetschel (1958) and by Sourreil, Beylot & Carrard (1969). As regards treatment, we intend to wait and see if spontaneous healing will occur.

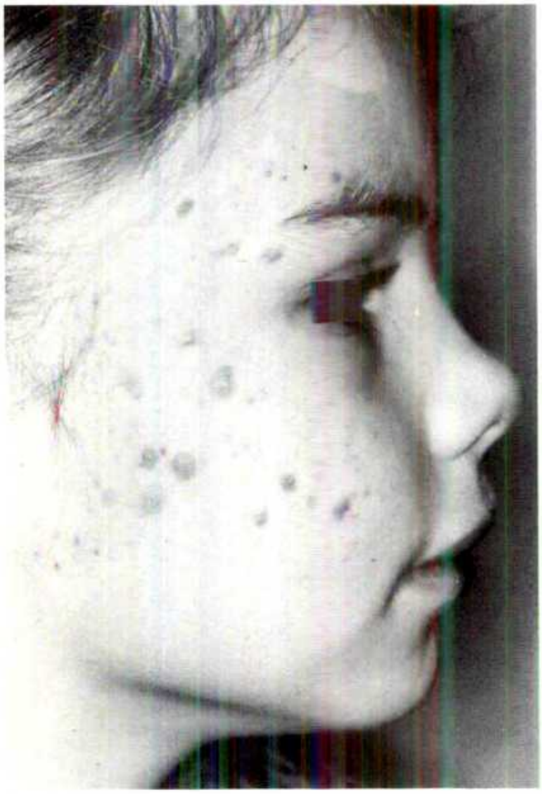

FIGURE 5. Hyperpigmented area with dark macules and reddish-brown papules.

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British Journal of Dermatology (1984) **110,** 121–126.

Correspondence

U.K. qualifying examination in dermatology

The interesting poll on dermatological training reported by Drs Dowd and Lovell (*British Journal of Dermatology* 1983, **109,** 235) raises the issue of a U.K. dermatological qualifying examination. We believe the case for such an examination has been misunderstood and requires restating. Since 1970 the recommendations of the Specialist Advisory Committee and the Committee for Postgraduate Medical Training of the B.A.D. have been followed, namely 3 years general medicine (after registration) and acquisition of the M.R.C.P. followed by 4 years of dermatology in one or more approved dermatological training centres. It was intended that these training centres and the trainees themselves be reviewed regularly to ensure adequate evaluation of each Senior Registrar.

Although strenuous efforts have been made, it is widely recognized that there is considerable unevenness in dermatological training between different centres, and exposure to some of the subspecialities of dermatology is minimal in some places.

An additional factor which has emerged is the big impact which advances in the biological sciences are now having on dermatology. Under the present scheme the products of the existing training systems are poorly equipped with the background knowledge to assimilate these advances into dermatological practice.

Although the majority of respondents to the Dowd and Lovell questionnaire feels that an examination is not necessarily the best way of assessing suitability for a consultant appointment, we consider that the most important advantage of such an examination is that it would do much to extend and improve the quality of dermatological training. It is proposed that the examination would be taken after successful acquisition of the M.R.C.P. at an appropriate stage or stages during the period of dermatological training, and should ideally be set up under the auspices of a Royal College.

It would have the following advantages:

(1) It would have a curriculum which in turn would encourage a structured training programme, to include training in basic science and internal medicine as related to the skin, pathology of the skin and clinical dermatology. It should be incumbent upon the Board responsible for the examination to organize a training programme. This should not be difficult if dermatological centres of excellence pool their resources nationwide. Thus, the examination would have the effect of greatly improving dermatological training at a time when there is considerable concern among junior staff about the quality of existing training facilities.

(2) It would ensure that successful applicants for consultantships in dermatology had reached an adequate level of training. This would be especially valuable in a speciality which has until recently had a shortfall between numbers of candidates for consultant posts and numbers of vacancies.

It could have some possible disadvantages. Some are mentioned in the Dowd and Lovell letter.

(1) Other specialities will 'jump on the bandwagon' and petition for their own specialist examination. Dermatology can plead a special case. It is perhaps somewhat further removed from general medicine than, say, cardiology and nephrology, and existing facilities for training are largely superior to our own in these subspecialities.

(2) It will detract from the research activities of dermatologists in training. This argument would be more persuasive if under the present conditions trainee dermatologists were busily engaged in research. But for the most part this clearly is not the case. In any event it is a reasonable assumption that broadening of their dermatological education will advance their motivation and ability to carry out research.

(3) There is also the problem of the fate of the candidate who fails the examination. Obviously this will be subject to much discussion, particularly on whether success in this examination should be mandatory. If this condition were imposed we feel it would probably be wise to take the examination in stages.

We appreciate that the concept of a qualifying dermatological examination is by no means new. But we feel that the nature of clinical practice of dermatology, its scientific basis and the increasingly exacting pressures which are being put upon dermatologists prompt an urgent reappraisal of the need for an examination. At any rate we hope this letter, together with that of Drs Dowd and Lovell will provoke constructive views on specialist training in dermatology and the best ways of improving it. We would propose further, the setting up of a working party to discuss these matters and would appreciate hearing from any dermatologist who may wish to participate.

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Structural skull defects in type IV Ehlers–Danlos syndrome

SIR, The Ehlers–Danlos syndrome (EDS) is a heterogeneous group of inherited disorders of connective tissue. The genetic, biochemical and clinical characteristics are so dissimilar that at least ten distinct variants of EDS have been identified so far (Hollister, Byers & Holbrook, 1982). Patients with type IV EDS do not show the marked skin hyperextensibility and joint laxity which were previously considered the hallmark of the disease. They have thin, fragile skin (through which a prominent venous network is readily visible) and mild joint hypermobility, generally limited to the digits. Rupture of the bowel and large arteries are the major causes of early mortality. This variety of EDS has an autosomal dominant inheritance, and is due to a lack or an abnormal synthesis of type III collagen, which is indispensable to the normal tensile strength of skin, vessels, uterus and gastro-intestinal tract (Pope *et al.*, 1975). We describe a case of type IV EDS who presented, besides the classical features of the disease, unusual structural defects of the skull, which predisposed to recurrent episodes of pneumococcal meningitis.

A 27-year-old man was admitted because of clinical evidence of meningitis. The diagnosis was confirmed by lumbar puncture, which yielded purulent cerebrospinal fluid, from which *Streptococcus pneumoniae* was isolated. Similar episodes had occurred elsewhere 5 and 20 months previously; on both occasions *S. pneumoniae* had been the responsible organism. In addition, since childhood he had suffered from repeated spontaneous dislocations of the right shoulder and easy bruising.

The patient showed thin, pale and only slightly hyperextensible skin; the underlying venous vasculature was visible. Joint hypermobility was limited to the right shoulder, which was dislocated, and to the digits; the distal phalanges of both thumbs were dislocated, while all the distal phalanges of the other finger and toes were subluxed. Atrophic scars were evident on his elbows and knees.

Review of his family revealed that in the space of four generations twelve subjects had been affected by

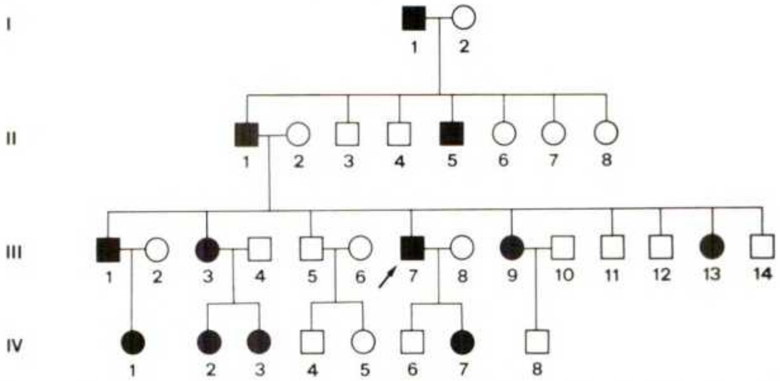

FIGURE 1. The pedigree of the family shows an autosomal dominant pattern of inheritance. Black squares and circles indicate affected males and females, respectively. The propositus is indicated by the arrow.

the same condition, with the characteristics of an autosomal dominant mode of inheritance (Fig. 1). The patient's father (II, 1) and one of his brothers (III, 1) died from gut perforation at age 48 and 27 years respectively; his grandfather (I, 1) and one uncle (II, 5) died suddenly of unknown causes at an early age. Besides the patient, seven affected members of the family are alive; one sister (III, 3) suffered from a stroke at age 8 and is still hemiparetic.

X-ray examinations showed a right-sided diaphragmatic eventration and multiple structural alterations

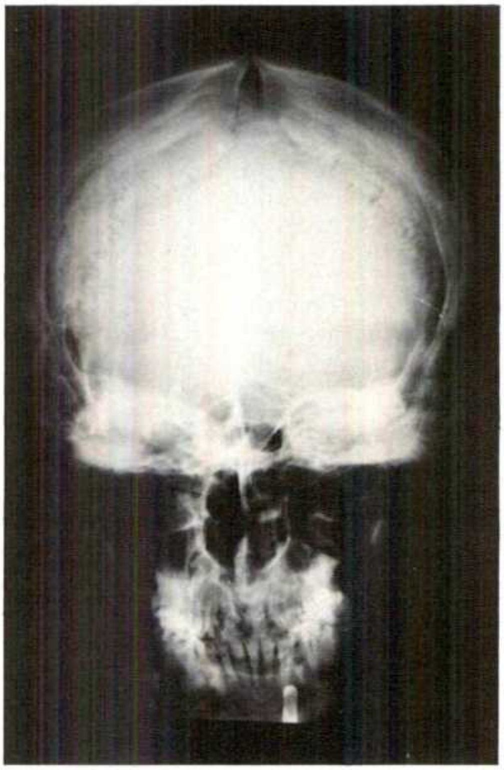

FIGURE 2. Anteroposterior skull film demonstrating dehiscence of the sagittal suture.

of the skull. A dehiscence of the sagittal suture (Fig. 2) and bony defects of the superior wall of the right maxillary sinus and of the ethmoid were shown.

Light and electron microscopy study of skin biopsy demonstrated abnormalities consistent with the clinical diagnosis of type IV EDS. A 2 weeks course of penicillin G intravenously ensured complete cure of the episode of meningitis.

Congenital or traumatic lesions of the skull are the constant predisposing factor of recurrent pneumococcal meningitis. In this case, potential portals of entry were found in the ethmoid and in the superior wall of maxillary sinus, although the defect in the sagittal suture appears to be less dangerous.

Although the clinical expression of type IV EDS is variable, the presence of structural skull abnormalities has not been reported previously. Since type III collagen is ubiquitously distributed in connective tissue, we can hypothesize that in our patient the inherited defective synthesis had produced the maldevelopment of skull bones in addition to the more typical stigmata. In fact, the disposition of hydroxyapatite crystals in bone formation requires the correct arrangement of normal collagen molecules (White *et al.*, 1977).

Patients with type IV EDS present a very high rate of surgical complications. Tissues are extremely fragile, rupture of vessels during operation is frequent and wound closure can be very difficult; neurosurgery, in this regard, obviously involves a most severe risk. For these reasons, the final decision concerning surgical repair of skull defects has been postponed, but we have instituted medical treatment (long-term prophylaxis with benzathine penicillin and antipneumococcal vaccination) keeping the patient under observation for the time being.

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An immunological mechanism for the reduced incidence of malignant melanoma in patients with acne?

SIR, In the paper on cutaneous factors related to the risk of malignant melanoma Beral *et al.* (*British Journal of Dermatology* 1983, **109,** 165) observed a negative correlation between the occurrence of acne and the development of malignant melanoma. The authors suggested that the reduced incidence of melanoma in acne patients might be explained by a reduction in transmission of ultraviolet radiation through the increased skin surface lipids characteristic of acne. Such an explanation appears logical enough, but surely it would apply only to those limited areas of skin (face, upper chest and back) protected by seborrhoea, and malignant melanoma developing at other sites ought not to be so influenced. It would be useful to know whether acne patients have fewer tumours just at high sebum sites.

Another possibility which might reduce the incidence of malignant melanoma in acne patients at both high and low sebum sites is immune enhancement, wrought by the greatly increased numbers of *P. acnes* on the skin of acne patients compared with non-acne controls (Leyden *et al.*, 1975). The *Corynebacteria* are potent stimulators of the reticulo-endothelial system as shown with *C. parvum* (Halpern *et al.*, 1963) and of complement pathways as shown with *P. acnes* (Webster & McArthur, 1982). Another *corynebacterium*, *C. granulosum*, has been successfully used to treat Fortner's melanotic melanoma in hamsters (Paslin, Dimitrov & Heaton, 1974). Subsequently, ten strains of *Corynebacteria* were compared for their effect on survival of C57Bl/6 mice bearing B16 melanomas. Seven of the strains caused significant prolongation of survival, and the most effective of these was *C. acnes*—*P. acnes* by present nomenclature (Paslin, 1977).

Thus, patients with acne harbour far greater numbers of *P. acnes* than do controls; *P. acnes* potently stimulates the immune system and has proven efficacy against experimental models of malignant melanoma; patients with acne would therefore be expected to have a reduced incidence of malignant melanoma. The data of Beral *et al.* (1983) are fully consistent with this view.

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Acquired cutis verticis gyrata due to rotational traction

SIR, We have recently seen an elderly patient who developed cutis verticis gyrata due to long matted hair with rotational traction.

A 70-year-old male sadhu ('holy man') attended our department with the complaints of itching, discharge and scaling in the left parietal region of the scalp for 5 months. He said that when he became a 'holy man' at puberty he had stopped cutting his hair and brought all the scalp hair over to the left side to make plaits. After some time, the hair in the plait became matted due to lack of combing and washing. To include the new growth of hair, he just rotated the plaits. On examination of the scalp, besides male pattern baldness we found superficial folliculitis, scaling, crusting and alopecia in the left parietal area. There were a few cutaneous folds resembling cutis verticis gyrata along the line of traction of the plaits. Each such fold corresponded to a plait.

Hair loss due to traction is not uncommon (Singh, 1975) but in this case the traction resulted not only in loss of hair but also in the development of cutaneous folds on the scalp. Rotational traction to the scalp had been continued for about 55 years and had resulted in clear-cut folds resembling cutis verticis gyrata.

In this case the plaits of hair measured 6½ feet (Fig. 1). This is very unusual in modern times, although it is nowhere near the maximum length of 26 feet recorded in an Indian sadhu (McWhirter & McWhirter,

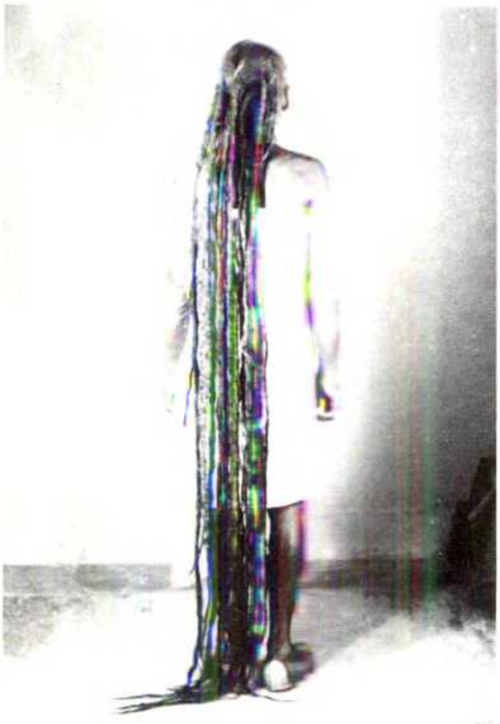

FIGURE 1. Long matted hair in a 'holy man'.

1974). As in the world record case, the length of the plait in our case dose not represent the actual length of single hairs because the hairs which have been shed during telogen have matted and contributed to the total length of the plait.

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Book Reviews

An Atlas of Skin Biopsy. R.P.Misra and I.D.Sanusi (1983) Springfield: C.C.Thomas. Pp. 110. Price $22.75.

In the preface the authors state that this book is intended for use as a bench reference for general pathologists interested in dermatopathology, post-graduate trainees and senior medical students who wish to have an update in immunodermatopathology. Unfortunately, I feel that this book will satisfy none of these groups of people adequately. It suffers from several important weaknesses.

Firstly, the illustrations are generally of poor quality and some are frankly out of focus. It tends to present data in a simplistic and rather uninspiring form with constant reiterations of lists of differential diagnosis. Finally, the lack of references is a grave defect.

It must be firmly stated that for anyone seriously involved in immunodermatopathology this book is far too over-simplified and lacks sufficient detail.

M.M.Black

Immunodermatology. Edited by I.N.Gigli, P.A.Miescher and H.J.Muller-Eberhard (1983) Berlin: Springer-Verlag. Pp. 183. Price D.M. 46.00.

This volume comprises thirteen papers selected from the journal *Springer Seminars in Immunopathology* and is intended to cover recent developments in immunopathology as applied to dermatology. The various authors, all from the U.S.A., provide good reviews of some clinical topics—dermatitis herpetiformis, erythema multiforme, urticaria, immune complex disease and atopic dermatitis. Also dealt with are more basic subjects such as mechanisms of immunologically mediated epidermal cell injury, the mast cell, chemotaxis, complement, photoxic reactions and the effect of UV radiation on contact hypersensitivity reactions. For anyone wishing to scan the field this is a useful collection.

J.J.Cream

Differential Diagnosis in Dermatopathology. A.B.Ackerman, J.Niven and J.M.Grant-Kels (1982) Philadelphia: Lea and Febiger. Pp. 195. Price U.S. $94.50.

A textbook of dermatopathology which highlights the differentiating features of cutaneous diseases which share many similar aspects is an invaluable educational aid and also provides a useful reference for the practising pathologist.

Beautiful colour photomicrographs illustrate in generous detail the important points of histological differentiation between a large series of pairs of conditions which frequently cause diagnostic confusion. Generally the choice of pairs for comparison represents those areas of dermatopathology which most frequently cause difficulty, although in certain instances such as 'psoriasis vs. nummular dermatitis', 'angio-lymphoid hyperplasia vs. angiosarcoma' and 'pyogenic granuloma vs. Kaposi's sarcoma', the idea of differential diagnostic uncertainty seems a little contrived and theoretical.

The excellent photographs are supplemented by a helpful explanatory text, although the authors' didactic viewpoint is occasionally over-prominent.

Best value from this book would be obtained by those who already have a working knowledge of dermatopathology; it is less suitable for the rank beginner and probably superfluous to the expert. It can be wholeheartedly recommended as an addition to departmental libraries and to dermatologists who wish to pursue an interest in dermatopathology; perhaps only this type of book can provide the subtle extra information that many conventional cutaneous pathology texts lack.

D.M.MacDonald

Virus Diseases and the Skin. T.W.E. ROBINSON and R.B. HEATH (1983) Edinburgh: Churchill Livingstone. Pp. 194. Price £15.00.

A slim book of fifteen chapters designed for 'dermatologists, ophthalmologists, venereologists, senior medical students and others whose duties take them into contact with patients who may have cutaneous viral infections' is by its very nature likely to have problems in apportioning the different sections. In general the authors, a dermatologist and a virologist, have produced an attractive and informative volume, well illustrated and very readable. However, they could have achieved a better balance of topics. Five chapters on general aspects of virus infections and ten on specific infections of the skin are probably about right, but the host response receives only five pages and, excellent condensation though it is, a more detailed treatment would have been better. The space could have been found by deletion of the recurring preoccupation with herpes simplex encephalitis, which accounts for more than one precious page in total and is unlikely to be the concern of those for whom the book is written. It also seem wasteful to devote ten pages to smallpox which was declared extinct in 1980. Anyone meeting a suspected case will surely seek expert help.

It would also seem more appropriate to deal a little more fully with herpes simplex problems in the immunodeficient, which are mentioned only briefly (p. [illegible]) and the scant references to acycloguanosine suggest that the book was a long time in preparation.

This delay could hardly account for the statement (p. 55) that 'up to 5% of cases of herpes genitalis are associated with Type 1 virus' as papers from Edinburgh and the U.S.A. in 1973 showed 13·3% and 13·4%, (Smith *et al.*, *Br. J. V.D.* **49,** 385; Kaufmann *et al.*, *Cancer Res.*, **33,** 1446) and since then figures of 43·4% have come from Japan (Kawan *et al.*, 1976; *Lancet*, **ii**, *Br. J. V.D.* **58,** 44). In the section on chickenpox no mention is made of the need to give zoster immune globulin on delivery to infants born of mothers with chickenpox in the 4 days preceding delivery—otherwise there can be a mortality of up to 30% (*Morbid. Mortal. Weekly Rept.* 1979, 589).

There are excellent chapters on measles and enterovirus infections. The rubella section is lucid but contains the misleading statement (p. 109) that single radial haemolysis is important in diagnosis, yet it was pointed out as early as 1975 and often subsequently that it does not detect specific IgM. In the absence of evidence that immunoglobulin prevents fetal infection is there any point in perpetuating administration of it to women who do not wish termination (p. 111)? And should termination be immediately recommended if pregnancy and rubella vaccination inadvertently coincide? The official opinion in the U.S.A. has moved against such a procedure as a routine (*Morbid. Mortal. Wkly. Rept.* 1981, **30,** 39) and congenital damage from current vaccines in this country has yet to be shown (Banatvala, *Lancet* 1981, **1,** 392).

Since cowpox is now known not to be primarily a disease of cows it seems a pity to state that a history of contact will suggest the diagnosis (p. 139). So it will, but reliance on such evidence of contact will fail to identify half the cases now occurring.

Despite these criticisms this is a useful introductory volume. It is to be hoped that it will see further editions and that these will enable the authors to make these changes they will surely want to make and to introduce new material such as the AIDS syndrome and the parvovirus story which have emerged whilst this first edition was in gestation.

J. NAGINGTON

Thesis received

Psoriasis—Therapy and Pathogenesis. P.C.M. VAN DE KERKHOF, University of Nijmegen, Javastraat 104, The Netherlands.

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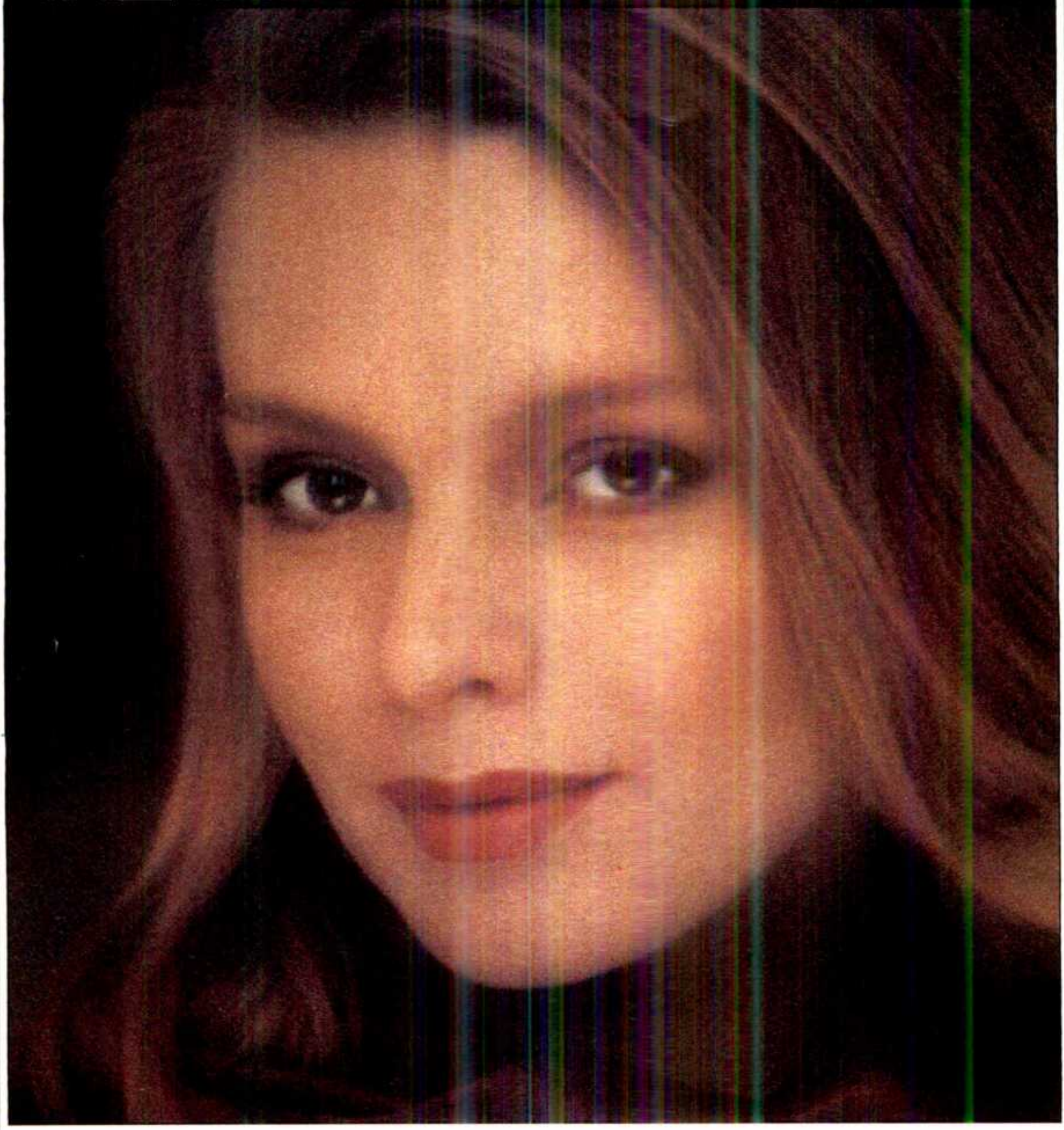

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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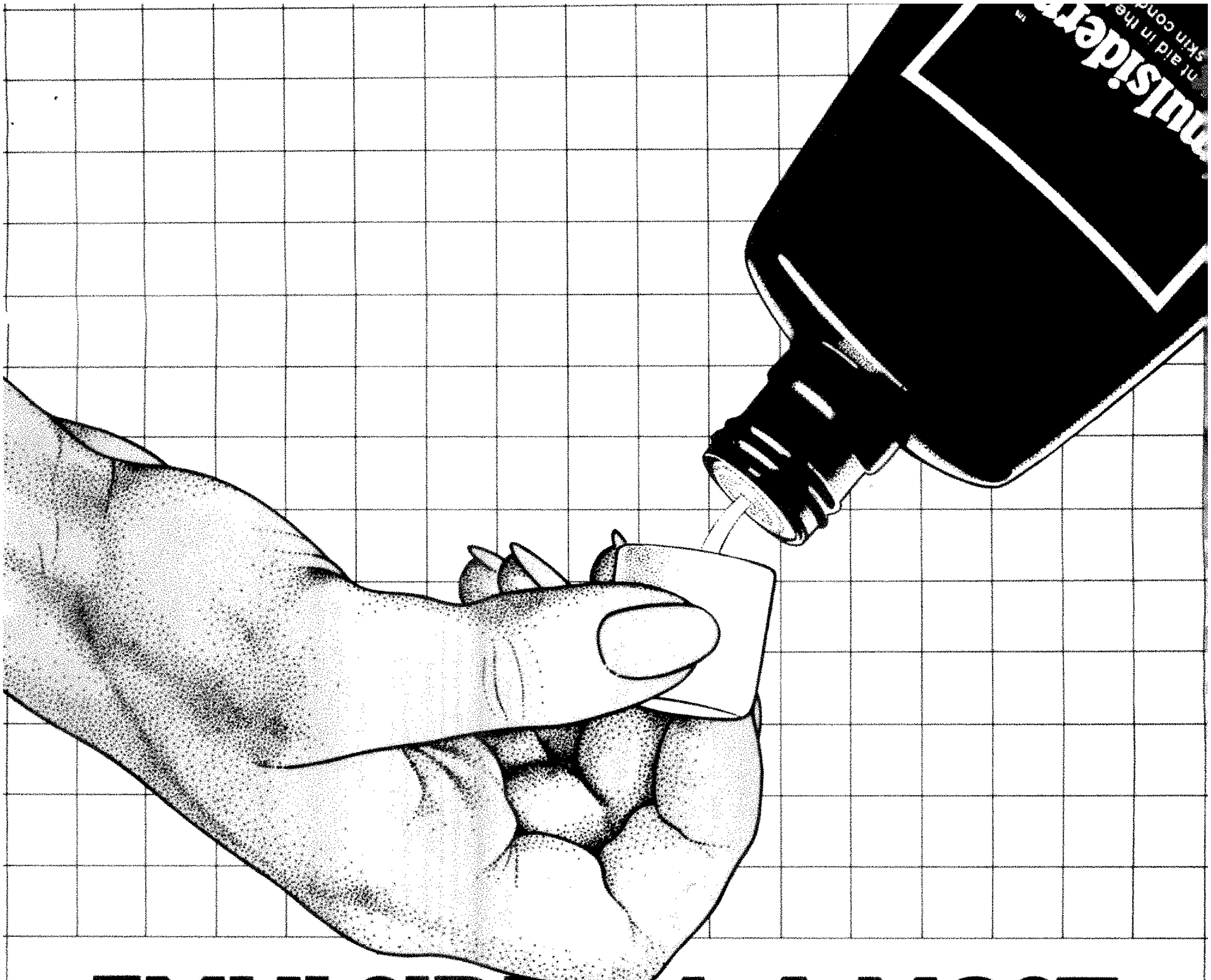

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Emulsiderm, a most effective measure against dry skin.

Presentation: Pale blue/green emulsion containing Benzalkonium Chloride BP 0.5%, Liquid Paraffin BP 25.0%, Isopropyl Myristate BPC 25.0%.
Uses: An aid in the treatment of dry skin conditions, especially those associated with eczema, ichthyosis or xeroderma.
Directions: Always shake bottle before use. In the bath: Add 2-3 capfuls to a 6-8 inch bath of warm water. For infant bathing use 1-2 capfuls. Soak for 5-10 minutes. Pat dry. On the skin: Rub a small amount of undiluted emollient into the dry areas of skin until absorbed.
Contra-indications, warnings etc:
Keep away from eyes. Take care to avoid slipping in the bath.
Package quantity: 250 ml polythene bottles with a 10 ml measuring cap.
PL 0173/0036. PA 278/8/1.
Further information is available from:
Dermal Laboratories Limited.
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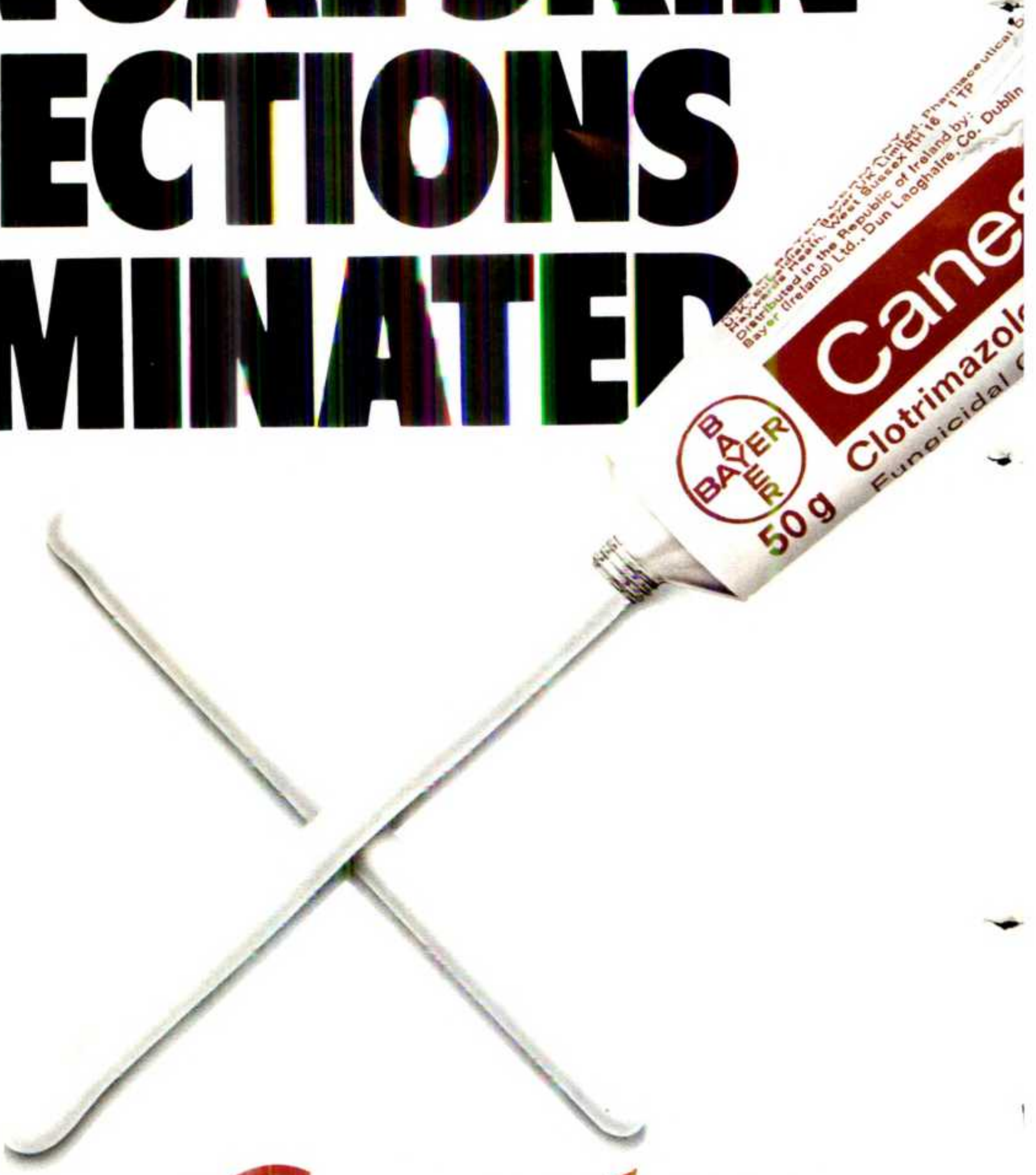
FUNGAL SKIN INFECTIONS ELIMINATED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Prescribing Information:
Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Solution* A clear solution containing 1.0% clotrimazole in polyethylene glycol. *Powder* A white powder containing 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections. **Dosage and Administration** *Cream, Atomiser Spray, Solution* Apply to the affected area 2-3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic against re-infection. **Side-effects** Rarely patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near naked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled. **Basic NHS costs** *Cream 20g* £1.84, *50g* £4.30. *Atomiser Spray 40ml* £5.17. *Solution 20ml* £2.40. *Powder 30g* £1.58. **Product Licence Numbers** *Cream* PL 0010/0016, PA21/4/2. *Atomiser Spray* PL 0010/0060, PA21/4/5. *Solution* PL 0010/0082, PA21/4/3. *Powder* PL 0010/0067, PA21/4/6.
Further information is available from

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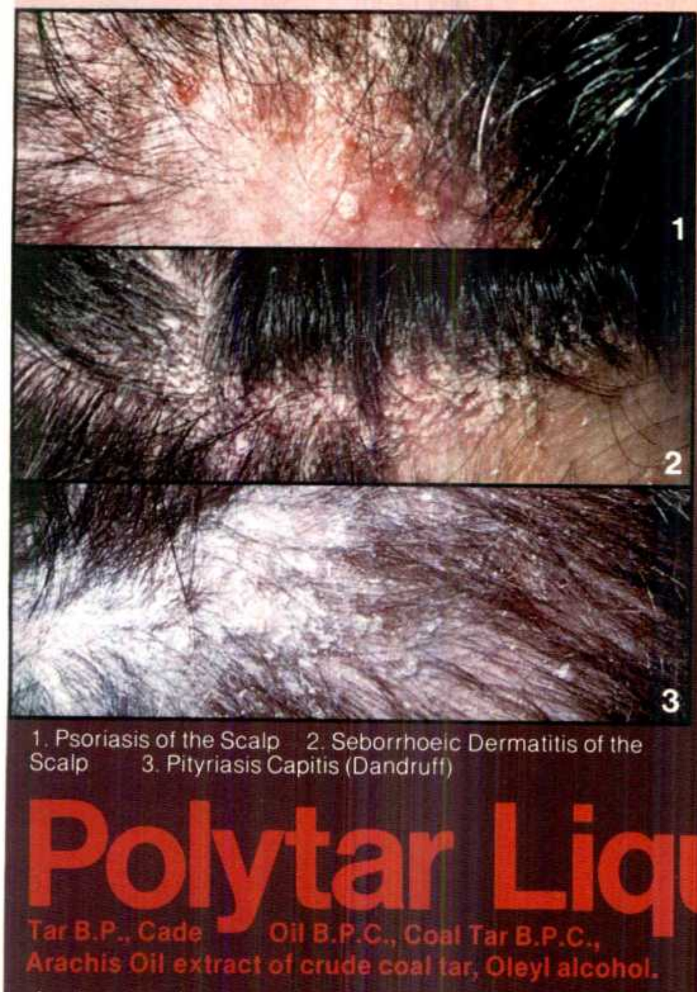
Common Disorders of the Scalp.



They all have one thing in common — the treatment

1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

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Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

ALSO Polytar Plus with protein

Prescribing Information.

Presentation Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coat Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.

Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	91p
	150ml	£1.34
	350ml	£2.37
	1 litre	£6.18
Polytar Plus	150ml	£1.43
	350ml	£2.80

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

Stiefel Laboratories (UK) Limited
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minocycline hydrochloride

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helps to minimise the risk of permanent scarring

Dosage Adults and children over 12 years. One tablet twice daily. Minocin may be taken with meals. Treatment of acne should be continued for a minimum of six weeks. **Contra-indications** Known tetracycline hypersensitivity. Systemic lupus erythematosus. **Precautions** Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. **Warnings and side effects** Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discoloration of teeth is a possible sequela of treatment during pregnancy or early childhood (up to 8 years of age). **Presentation** Beige tablets containing 50mg of minocycline as the hydrochloride, supplied in bottles of 100 at a basic NHS cost of £22.44 . PL 0095/0062. MINOCIN is a trademark. Full prescribing information is available on request.

Lederle Laboratories Division of Cyanamid of Great Britain Ltd, Fareham Road, Gosport, Hants PO13 0AS (Tel: 0329-236131)

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Betnovate RD

(betamethasone valerate)

Simply Betnovate, Ready Diluted

Prescribing information

Presentation

Betnovate RD Cream and Ointment contain betamethasone valerate ready-prepared in a one-in-four dilution, giving a strength of 0·025%.

Uses

Inflammatory dermatoses responsive to topical corticosteroid therapy. Betnovate RD preparations are indicated for maintenance treatment when control has been achieved with Betnovate (0·1%) Cream or Ointment.

Dosage and administration

A small quantity of Betnovate RD should be applied to the affected area two or three times daily. Betnovate RD Cream is especially appropriate for moist or weeping surfaces and Betnovate RD Ointment for dry, lichenified or scaly lesions, but this is not invariably so.

Precautions

Long-term continuous topical therapy should be avoided where possible, particularly in infants and children, as adrenal suppression can occur even without occlusion. Appropriate antimicrobial therapy should be used whenever treating inflammatory lesions which have become infected. Any spread of infection requires withdrawal of topical corticosteroid therapy and systemic administration of antimicrobial agents. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Prolonged and intensive treatment with highly active corticosteroid preparations may cause local atrophic changes in the skin such as striae, thinning and dilatation of the superficial blood vessels. As with other topical corticosteroids, prolonged use of large amounts, or treatment of extensive areas, can result in sufficient systemic absorption to produce the features of hypercorticism. This effect is more likely to occur in infants and children. Betnovate RD preparations are usually well tolerated, but if signs of hypersensitivity appear, application should stop immediately.

Contra-indications

Rosacea, acne and peri-oral dermatitis. Skin lesions caused by infection with viruses (e.g., herpes simplex, chickenpox), fungi (e.g., candidiasis; tinea) or bacteria (e.g., impetigo). Hypersensitivity to the preparation.

Product licence numbers

Betnovate RD Cream: 0004/0277
Betnovate RD Ointment: 0004/0278

Basic NHS cost (exclusive of VAT)

Betnovate RD Cream/Ointment
100 gram tubes £2·90

Glaxo

Leaders in topical steroid therapy

Further information on Betnovate and Betnovate RD is available on request from Glaxo Laboratories Limited
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Betnovate is a Glaxo trade mark

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T/Gel
Shampoo
Relieves itching, flaking scalp with regular use.
Aids in the treatment of scalp psoriasis, dandruff and seborrheic dermatitis.
125 ml
Neutrogena®

T/GEL is a new medicated shampoo formulated as effective everyday therapy for scalp psoriasis, dandruff and seborrheic dermatitis. Regular use helps control itching, flaking and scaling while leaving the hair lustrous and manageable. T/GEL contains Neutar, a coal tar extract, equivalent in potency to 2% crude tar in suppressing D.N.A. synthesis (cell turnover). The unique clear T/GEL formula gives a rich lather, is pleasant to use, and free from lingering tar odour, encourages patient compliance.

PRESCRIBING INFORMATION

PRESENTATION T/GEL is a concentrated medicated tar based shampoo containing Neutar a solubilised coal tar extract 2% in a bland shampoo base consisting of: purified water, USP; sodium laureth sulfate; cocamide DEA; cocamidopropyl betaine; fragrance; imadazolidinyl urea; methylparaben; propylparaben; tetrasodium EDTA and citric acid.

USES Indicated as an effective aid in the treatment of psoriasis, dandruff and seborrheic dermatitis.

DOSAGE AND ADMINISTRATION Hair should be wetted thoroughly and a liberal amount of T/GEL applied and massaged into scalp, allow lather to remain on scalp for several minutes, then rinse; repeat application and rinse thoroughly.

PACK AND PRICE Pack 125 ml. Basic NHS price £1.52

PRODUCT & LICENCE NUMBER 5165/0001

Neutrogena®

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Neutrogena (U.K.) Ltd., P.O. Box 27,
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Because it's strong . . .

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Because it's strong . . .

it often gives prolonged periods of remission.

> **❛Thus, using a potent steroid for a short duration is usually safe and often produces results, whereas using a weak topical steroid indefinitely does not.❜**

Fry L. Br Med J 1982; 284:1093—1

Dermovat

(clobetasol propionate)

Its strength is its strength

Prescribing information

Dosage and administration
Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

Side effects
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also, prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing.

Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications
Rosacea, acne and peri-oral dermatitis. Bacterial, fungal or viral diseases of the skin. Hypersensitivity to the preparation.

Basic NHS cost
(exclusive of VAT)
Dermovate Cream or Ointment 25 gram tubes £2.07 (al available in 100 gram tubes).

Presentation	**Product licence number**
Dermovate Cream:	4/0219
Dermovate Ointment:	4/0220

Glaxo Leaders in topical steroid therapy

Further information on Dermovate is available from:
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Dermovate is a Glaxo trade mark

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Abstract forms for the 18th Scientific Meeting will be available from the Honorary Secretary and the Local Organizer after July 1983. Abstracts can be submitted from non-Members of the Society. The deadline for receipt is 23 November 1983.

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betamethasone valerate

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The preparation has an immediate cooling effect and gives rapid relief from the itching and inflammation associated with seborrhoeic dermatitis and other troublesome scalp conditions.

Betnovate Scalp Application is colourless, odourless and does not stain or leave unsightly residue in the hair.

Specially formulated; specially packed to make treatment easy

Prescribing information

Dosage and administration
Apply sparingly night and morning until improvement occurs. With Betnovate Scalp Application, improvement may be sustained by applying once a day or less frequently.

Contra-indications
Viral and dermatophyte infections of the scalp. Hypersensitivity to the preparation.

Precautions
Care must be taken to keep the preparation away from the eyes. Do not use near a naked flame. In infants and children avoid long term continuous topical therapy where possible, as adrenal suppression can occur even without occlusion. In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects
Betnovate Scalp Application is usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. With all topical corticosteroids, when extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism. This is more likely to occur in infants and children with occlusion, or when treatment is prolonged. Local atrophy may occur after prolonged treatment under occlusion. Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Basic NHS cost (exclusive of VAT)
30ml – £1·70

Product Licence number
4/5133

Further information on Betnovate (trade mark) Scalp Application is available from: Glaxo Laboratories Limited Greenford, Middlesex UB6 0HE

Glaxo

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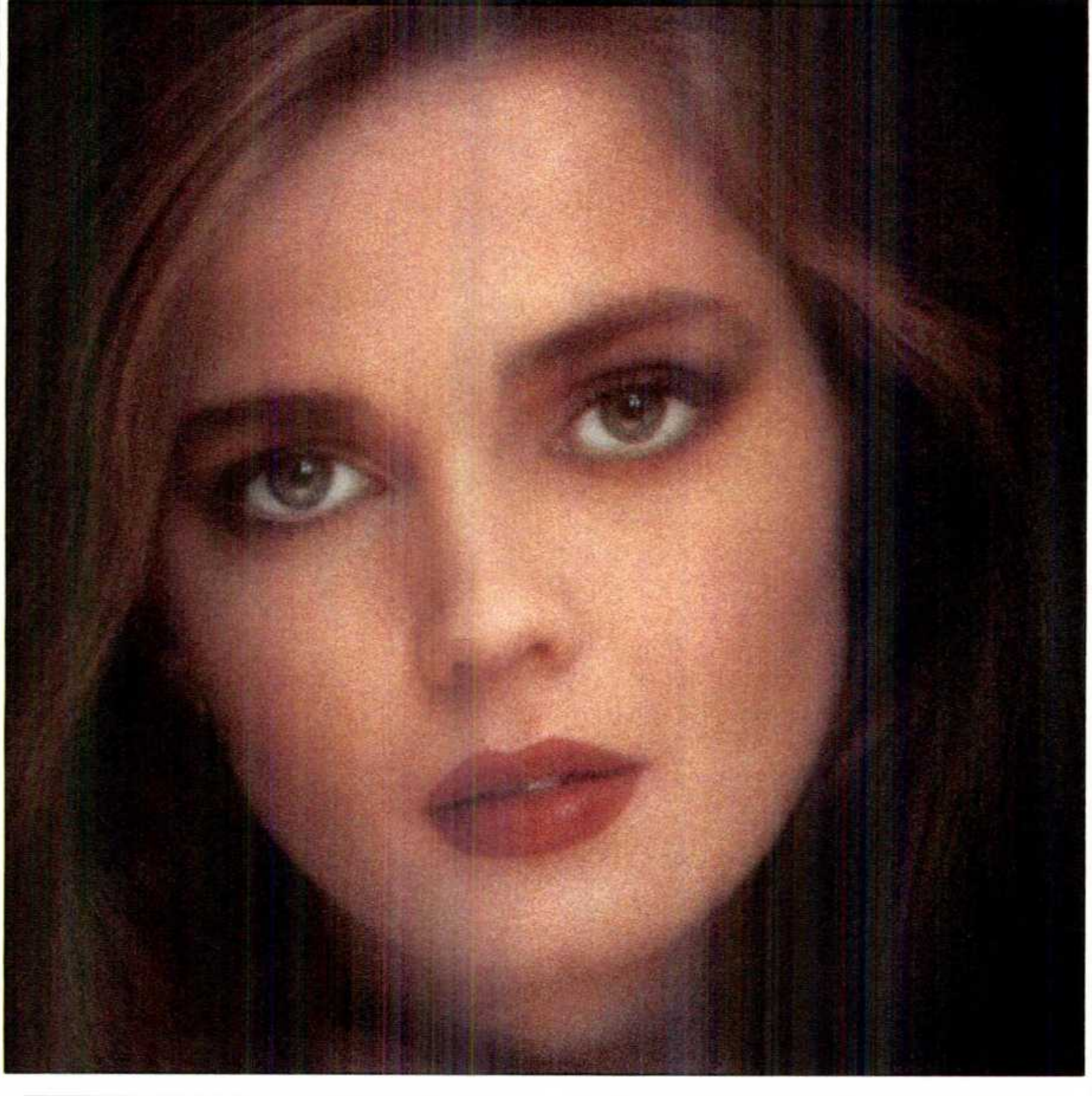


ACCEPTABLE TO YOU

In chronic psoriasis, Alphosyl offers the effectiveness of coal tar with its direct antiproliferative action on epidermal cells, together with the benefits of allantoin which helps remove plaque scales.

Alphosyl, available as a Cream, Lotion and Shampoo (Alphosyl Application PC) is therefore the ideal maintenance therapy for most types of chronic psoriasis.

ACCEPTABLE TO PATIENTS

Unlike crude coal tar preparations, Alphosyl is easy to apply, has an unobtrusive smell, and is formulated to minimise staining.

Additionally, unlike dithranol, Alphosyl will not damage healthy skin. This combination of effectiveness and cosmetic acceptability encourages patient compliance and simplifies the management of chronic psoriatic conditions.

ALPHOSYL CREAM AND LOTION

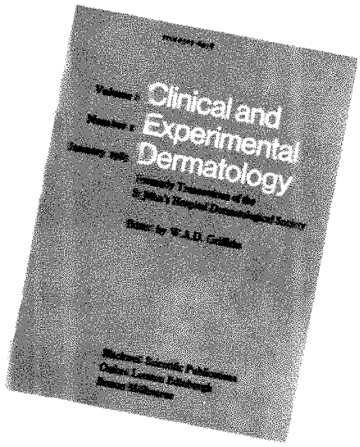
(coal tar 5%, allantoin 2%)

FULLER FREEDOM FROM PSORIASIS

Presentation LOTION AND CREAM. Containing refined alcoholic extract of coal tar 5%, allantoin 2% in a greaseless vanishing-cream base. The lotion is a light free-flowing emulsion and the cream is smooth and homogeneous, light tan in colour. **Uses** Dermatological stimulant for the treatment of psoriasis. **Dosage and administration** Apply liberally two to four times daily, massage well into the affected areas. Occlusive dressings are unnecessary. **Contra-indications, warnings etc.** For external use only. Discontinue if irritation occurs or in cases of sensitivity to coal tar. **Pharmaceutical Precautions** Do not refrigerate. Store at room temperature. **Legal category P. Package quantities/Basic NHS Cost** ALPHOSYL LOTION. Bottle of 250ml., £2.31. ALPHOSYL CREAM. Tube of 75g., £1.59. **Further information** Alphosyl Lotion is the basic treatment for psoriasis of the body and scalp. Alphosyl Cream is designed especially for lubrication in the intertriginous areas. **Product Licence Nos.** Alphosyl Lotion 0036/5008. Alphosyl Cream 0036/5006. Further information is available on request. Stafford-Miller Ltd., Professional Relations Division, Hatfield, Herts. AL10 0NZ.

Clinical and Experimental Dermatology

Formerly Transactions of the St John's Hospital Dermatological Society



Clinical and Experimental Dermatology publishes original articles covering all aspects of the normal biology of the skin and clinical and experimental dermatology. Regular features include the full texts of bi-annual orations by speakers of international renown, symposia on topics of major dermatological interest, and Society clinical proceedings. Invited review articles, book reviews and correspondence are published occasionally. The journal is the official organ of the St John's Hospital Dermatological Society, but it attracts contributions from all countries and its circulation is equally international.

Manuscripts for publication should be sent to the Editor, Dr W.A.D. Griffiths, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ, U.K.

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ACCEPTABLE TO PATIENTS

Unlike many alternative treatments, Alphosyl HC has an unobtrusive smell and is formulated in a vanishing cream base to minimise staining.

Its combination of effectiveness and cosmetic acceptability encourages patients to comply with your prescription.

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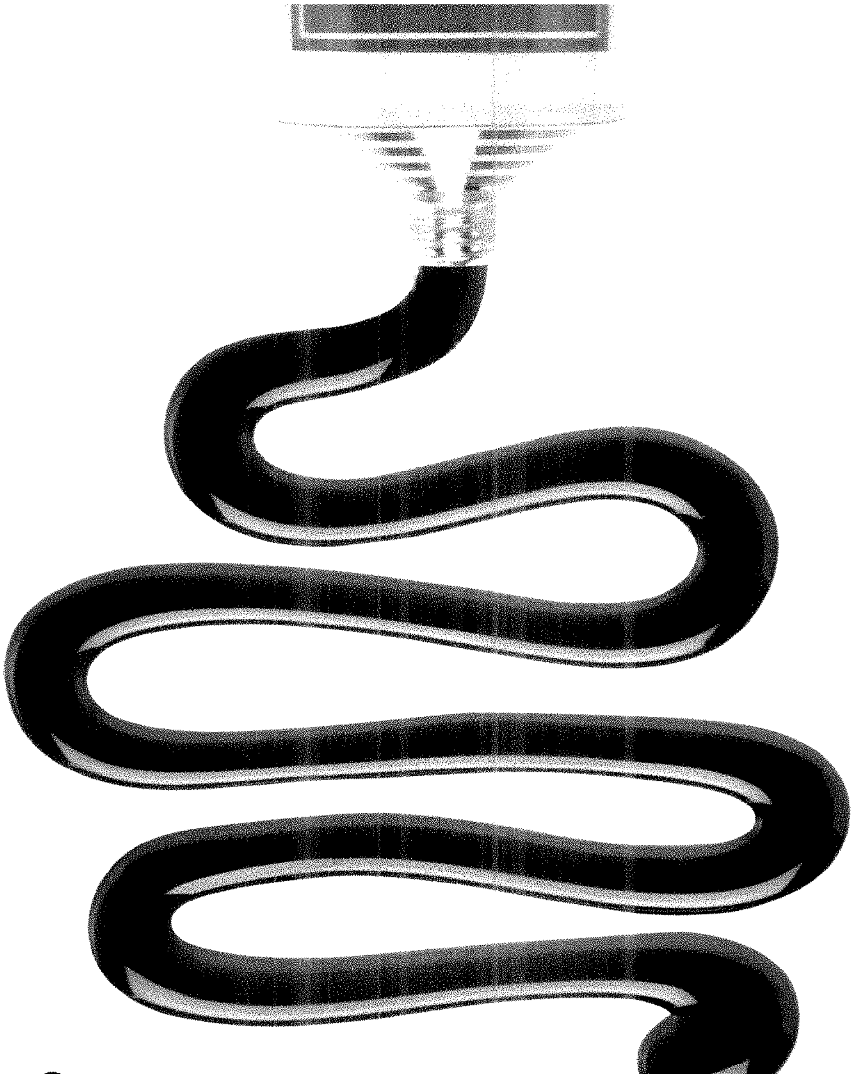
(coal tar 5%, allantoin 2%, hydrocortisone 0.5%)

FULLER FREEDOM FROM PSORIASIS

Presentation A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone PhEur 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogeneous and is light tan in colour. **Uses** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage and administration** Apply two to four times daily, massaging well into the affected areas. **Contra-indications, warnings etc.** In infants long-term continuous therapy should be avoided. Adrenal suppression can occur even without occlusion. Topical administration of corticosteroids to pregnant animals can cause abnormality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. Do not refrigerate. **Legal category POM. Package quantities** Tubes of 30g. and 45g. **Basic NHS cost** £1.73 for 30g., £2.46 for 45g. **Further information** Alphosyl HC cream is intended for the treatment of the acute stages of psoriasis when the basic Alphosyl action is enhanced by the addition of hydrocortisone. **Product Licence No.** 0036/0026. Further information is available on request. Stafford-Miller Ltd., Professional Relations Division, Hatfield, Herts. AL10 0NZ.



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Prescribing Information

Use: An aid in the topical treatment of venous and other types of skin ulcers.
Directions: Clean and dry the ulcer site, apply and smooth the Variclene gel over the affected area. Cover with a sterile dressing or pad and, where appropriate, apply compression bandaging. Repeat as required at intervals of not more than seven days.
Contra-indications, Warnings, etc: Avoid contact with clothing and linen. Occasionally, transient stinging on application may occur. In cases of arterial or extensive, painful venous ulceration, this may be prolonged, when treatment should be discontinued until greater tolerance has been achieved. Keep away from the eyes.
Presentation: 50g tube **Product Licence:** 0173/0015 (PA 278/10/1)

Variclene®

Green aqueous gel containing 0.5% w/w Brilliant Green BP, 0.5% w/w Lactic Acid BP.

Variclene is a registered trade mark

British Journal of Dermatology (1984) **110**, 129–138.

Clinical and Laboratory Investigations

Relationship of solar keratosis and history of skin cancer to objective measures of actinic skin damage

C.D.J.HOLMAN*, B.K.ARMSTRONG*, P.R.EVANS*, G.J.LUMSDEN*, K.J. DALLIMORE†, C.J.MEEHAN†, J.BEAGLEY‡ AND I.M.GIBSON‡

*NH and MRC Research Unit in Epidemiology and Preventive Medicine, †Department of Medicine, University of Western Australia, Perth & Department of Dermatology, Royal Perth Hospital, Perth and ‡School of Biology, Western Australia Institute of Technology, Perth

Accepted for publication 24 July 1983

SUMMARY

Two methods for the assessment of severity of actinic skin damage were evaluated in a population-based survey of 1216 subjects. After controlling for the effects of age, skin texture changes graded by cutaneous microtopography were found to be associated strongly with the presence of solar keratoses and a past history of non-melanotic skin cancer. Changes in skin condition graded by paraocular photography had a weaker relationship with the presence of keratoses and showed no association with non-melanotic skin cancer. These results, together with a higher level of agreement between observers in grading cutaneous microtopographs, support the use of cutaneous microtopography as an index of actinic skin damage in epidemiological research.

The role of sunlight in the causation of cancer of the skin has been studied for more than 100 years (Thiersch, 1875), and today ultraviolet radiation (UVR) from the sun is generally accepted as the dominant risk factor, at least in the development of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) in fair-skinned races (Scotto & Fraumeni, 1982). In Australia, New Zealand and the United States of America, preventive programmes aimed at a reduction in sun exposure are common (Smith, 1979; Hansen, 1980) but at present the effects of these programmes are uncertain.

At least two benefits could follow from the development of objective methods of assessing the severity of skin changes caused by UVR. First, if highly correlated with past sun exposure, they would reduce dependence on subjective questionnaires for measurement of past sun exposure. This would be especially valuable in studies of malignant melanoma, a disease which, unlike

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BCC and SCC, shows inconsistencies in its relationship to apparent sun exposure (Lee, 1982). Second, it would be reasonable to assume that early actinic skin damage is much more frequent than, and precedes the development of, skin cancer (although perhaps not all types of melanoma), by many years. Thus, measurement of early actinic changes may provide a more rapid and more statistically powerful method of evaluation of effectiveness in studies of control of sun exposure, than would be possible by monitoring the incidence of skin cancer.

In this paper we describe two previously devised techniques that allow a graded assessment of changes in the skin thought to result from sun exposure, evaluate their reliability and describe their relationship to solar keratosis and past history of skin cancer.

Origins of cutaneous microtopography and paraocular photography

The use of silicone rubber impression materials to make a mould of the skin's surface, followed by the casting of an Araldite replica, was initially developed as an aid to study of the anatomy of normal skin and its accessory structures (Sarkany, 1962; Barnes, 1973). In 1979, Makki, Barbenel & Agache described a quantitative method of assessment of skin roughness using a surfometer and Araldite skin surface replicas made from silicone rubber moulds. They suggested that their technique might prove useful in the study of accelerated ageing of the skin due to exposure to UVR. More recently, Beagley & Gibson (1980) have devised a system of visual grading of skin surface moulds, using a low power dissecting microscope. Their method, which we refer to as cutaneous microtopography (CMT), is well suited for use in surveys of large numbers of subjects, because it obviates the need for sophisticated and time-consuming equipment, and does not require Araldite replicas of the skin moulds.

The system of grading cutaneous microtopographs described by Beagley & Gibson (1980) relates to changes in skin surface texture, which in normal skin, is composed of a series of transverse and diagonal *primary lines*, which intersect to form quadrilaterals and triangles. Within these primary figures are sets of smaller *secondary lines* which often meet in the centre of the figure, forming a star configuration. The six-step grading system devised by Beagley and

TABLE 1. The Beagley–Gibson system of grading cutaneous microtopographs taken from the dorsum of the hand

Grade	Features
1	Primary lines are all of the same depth. Secondary lines are all clearly visible, are nearly the same depth as the primaries, and often meet to form an apex of triangles ('star formation').
2	Some flattening and loss of clarity of the secondary lines. Star formations are still present, but often one or more of the secondary lines making up the configuration are unclear.
3	Unevenness of the primary lines. Noticeable flattening of the secondaries with little or no star formation.
4	Macroscopic deterioration in texture. Coarse, deep primary lines. Distortion and loss of secondary lines.
5	Noticeable flat skin between the primary lines. Few or no secondary lines.
6	Large deep and widely spaced primary lines. No secondary lines.

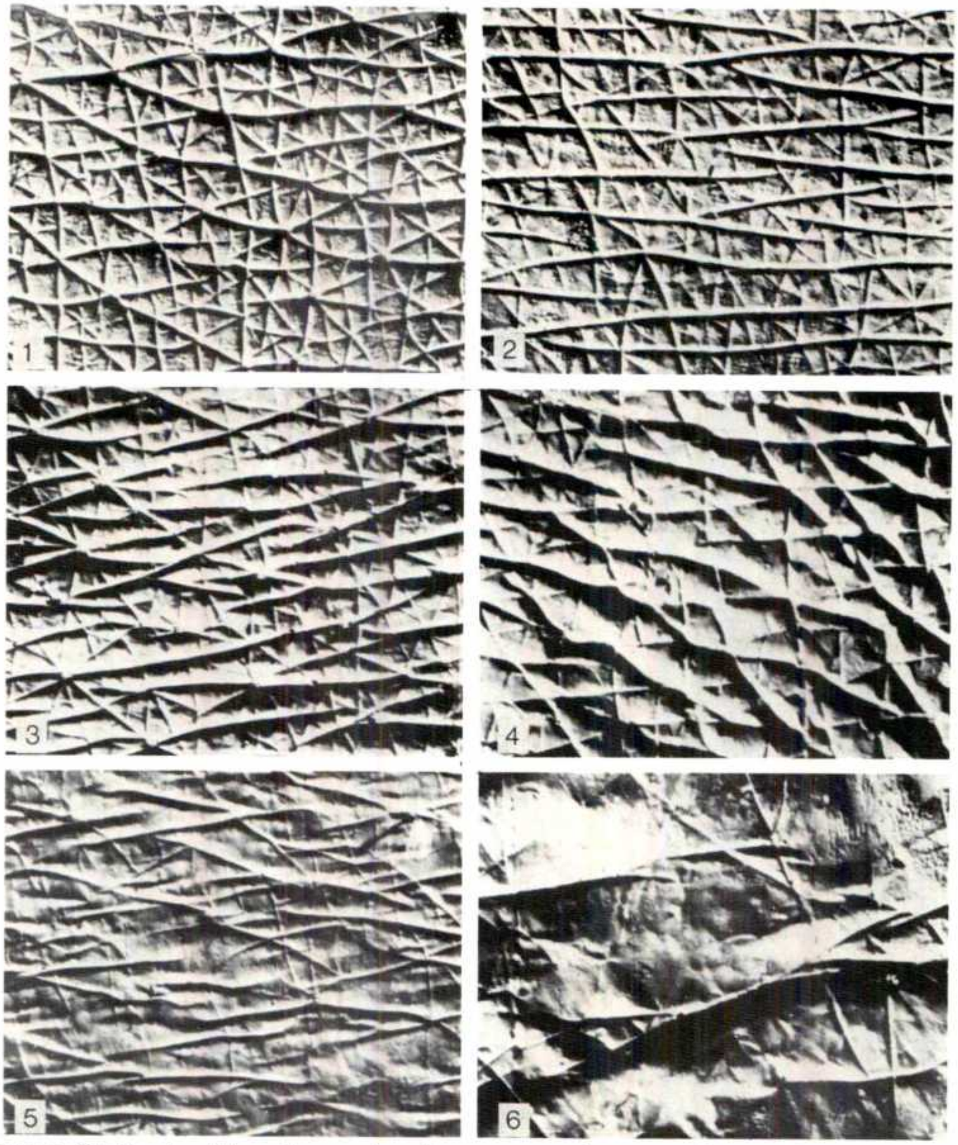

FIGURE 1. The Beagley–Gibson System of grading cutaneous microtopographs (magnified × 6), showing photographs typical of the six grades.

Gibson, and based on alterations in these skin surface characteristics thought to reflect actinic damage, is summarized in Table 1 and illustrated in Fig. 1. (Further details are available from Dr I.M.Gibson, School of Biology, Western Australia Institute of Technology, Hayman Rd., Bentley, 6102, Western Australia.)

A method of visual grading of facial skin wrinkling was developed by Daniell (1971) to test the hypothesis that 'crow's feet' are more common and more severe in cigarette smokers than in non-smokers. 'Crow's feet' (wrinkles in the lateral peri-orbital area) have been thought also to be caused by sun exposure, the ageing process and other factors such as massive weight

TABLE 2. The Daniell system of grading paraocular photographs

Grade	Features
1	Essentially unwrinkled. Two or three shallow wrinkles less than 1·5 cm in length may be present.
2	Several wrinkles, each of which may be 3 cm long. The number of significant wrinkles may be between two and six.
3	Several prominent wrinkles 3 to 4 cm long. Many smaller wrinkles may be present and may involve the forehead skin, but not the cheek.
4	Wrinkles extend superiorly and inferiorly, usually 5 cm or more, and involve the cheek area. Wrinkles of unusual depth may be 4 cm long.
5	Wrinkles extend from the 'crow's foot' area and are prominent over the cheek and forehead.
6	Profound wrinkling extending over most of the face.

reduction. Daniell applied his method by inspection of live subjects and by reviewing photographs taken from the left 'crow's foot' area of their faces; the latter we have called paraocular photography (POP). The six-step grading system of Daniell is summarized in Table 2; illustrations of the various grades were published in Daniell's original paper (Daniell, 1971).

MATERIALS AND METHODS

To further examine the use of CMT and POP as indices of actinic skin damage, advantage was taken of the 1981 Busselton Population Survey. Busselton is a coastal town situated 200 km south of Perth, the capital of Western Australia, and has an adult population of approximately 7000 persons. Since 1966 it has been the location of a triennial health survey in which all electors resident in the surrounding electoral subdivisions are invited to participate (Curnow *et al.*, 1969).

Between 23 November and 3 December 1981 a total of 1216 Busselton Survey attenders (35% of the total attendance of 3445 subjects) were examined and formed the basis for the present investigation. The subjects were selected in random blocks of fifteen based on their time of arrival at the Survey Centre, and comprised 560 males and 656 females aged 16 to 86 years. The following data were collected:

(i) a questionnaire including a question on *past treatment for non-melanotic skin cancers* (BCC or SCC);

(ii) the presence and number of *solar keratoses* on the face, ears and backs of the hands as assessed by a dermatologist in training (K.J.D. or C.J.M.);

(iii) a *cutaneous microtopograph* taken from the dorsum of the left hand, and later graded, without knowledge of any other characteristics of the subjects, by the method of Beagley & Gibson (Table 1), using a binocular dissecting microscope at × 10 magnification;

(iv) a *paraocular photograph* taken from the left side of the face, and later graded, also under blind conditions, by the method of Daniell (Table 2).

For the purpose of the survey, a solar keratosis was defined as a macroscopic, discrete, keratotic skin lesion with at least some erythema. Scars from treated keratoses were not enumerated.

The silicone rubber impression material used to produce cutaneous microtopographs was Optosil Flussig® (Bayer Leverkusen, West Germany), a viscous white liquid which sets in 3 to 5 minutes after addition of a catalyst. When applied, the liquid flows around projections arising from the skin surface, including hair shafts, which become embedded in it. Even in very hirsute men, removal of the cured microtopograph can be accomplished without causing pain or depilation, provided that it is stripped slowly and steadily from the skin surface.

Black and white paraocular photographs were taken with a Kodak Pocket Instatech Close-up Camera (No. 1P, Lester A. Dine Inc., New York), which has a frame to ensure standardization of lighting and distance. The area of facial skin included in each photograph measured 7 cm by 7 cm. The survey data were processed by computer using the Statistical Package for Social Sciences (Nie *et al.*, 1975). The strength and significance of relationships between study factors were analysed by use of methods described by Mantel (1963).

Skin moulds and photographs taken from the last fifty subjects examined were chosen for an assessment of inter-observer variation in the use of the grading systems. Each of the selected microtopographs and photographs was graded independently by the two observers (P.R.E. and G.J.L.) who had shared the task of grading all of the survey material. Agreement levels were calculated by the method of Fleiss, Nee & Landis (1979) using kappa statistics.

TABLE 3. Distribution of subjects according to number of solar keratoses identified at the Busselton Population Survey, 1981, by sex and 10-year age group

	Number of solar keratoses				
Sex and age group	None	1–4	5–9	10–14	15+
Males					
16–19	11 (92)[a]	1 (8)	—	—	—
20–29	75 (94)	5 (6)	—	—	—
30–39	58 (71)	19 (23)	3 (4)	1 (1)	1 (1)
40–49	49 (55)	24 (27)	13 (15)	3 (3)	—
50–59	55 (51)	32 (30)	7 (7)	6 (6)	7 (7)
60–69	27 (24)	36 (32)	20 (18)	10 (9)	20 (18)
70–79	17 (25)	12 (17)	12 (17)	13 (19)	15 (22)
80–86	2 (25)	5 (62)	—	—	—
All ages	294 (52)	134 (24)	55 (10)	33 (6)	44 (8)
Females					
16–19	13 (100)	—	—	—	—
20–29	82 (99)	—	1 (1)	—	—
30–39	98 (87)	14 (12)	1 (1)	—	—
40–49	73 (76)	17 (18)	2 (2)	4 (4)	—
50–59	71 (53)	37 (28)	14 (11)	7 (5)	4 (3)
60–69	61 (40)	47 (31)	18 (12)	7 (5)	18 (12)
70–79	20 (39)	11 (22)	9 (18)	4 (8)	7 (14)
80–86	4 (25)	4 (25)	2 (12)	3 (19)	3 (19)
All ages	422 (64)	130 (20)	47 (7)	25 (4)	32 (5)

[a] Row per cent in parentheses.

RESULTS

The distributions of male and female subjects in 10-year age groups, according to the number of solar keratoses present on the face, ears and hands are shown in Table 3. Forty-eight per cent of men and 36% of women had at least one keratosis at the time of the survey, whereas ten or more keratoses were observed in 14% and 9% of the sexes respectively. The prevalence of solar keratosis increased sharply with age. At ages 20–29 years only 4% of subjects had keratoses, compared with 75% of those aged 80–86 years. A past history of treatment for non-melanotic skin cancer was reported by 8% of the study group as a whole, and by 22% of men and 10% of women in the 70–79 year (i.e., average life expectancy) age group. The population of Australia is well known as having a very high incidence of skin cancer (Gordon *et al.*, 1972). Although we could find no population-based data on the prevalence of solar keratoses in other countries, our results suggest that solar keratoses are extremely common in Australia, affecting a majority of people at some time in their life.

The relationships of CMT and POP to the presence of solar keratoses and history of non-melanotic skin cancer are summarized in Tables 4 and 5. Solar keratoses were very strongly related to microtopograph grades, such that after control of age by stratification into 5-year age groups, a subject with a grade 6 microtopograph was 44·6 times more likely to have a keratosis than a subject with a microtopograph graded as 1 or 2. (From Table 4, note that no subject with a

TABLE 4. Relationships of cutaneous microtopography and paraocular photography to the presence of solar keratoses (SK) in the Busselton Population Survey, 1981

	Solar keratoses			After control of age in 5-year groups	
	Present	Absent			
Variable and grade	No. of subjects (%)	No. of subjects (%)	Crude odds ratio	Odds ratio	Significance of trend
Cutaneous microtopography[a]					
Grade 1	0 (—)	24 (3)	1·0	1·0	
Grade 2	2 (< 1)	67 (10)			
Grade 3	15 (3)	178 (25)	3·8	1·5	
Grade 4	82 (16)	188 (27)	19·8	8·5	
Grade 5	179 (36)	181 (26)	45·0	15·7	
Grade 6	218 (44)	70 (9)	141·7	44·6	< 0·00001
Paraocular photography[b]					
Grade 1	20 (4)	177 (25)	1·0	1·0	
Grade 2	141 (28)	265 (37)	4·7	1·4	
Grade 3	153 (31)	172 (24)	7·9	2·4	
Grade 4	108 (22)	66 (9)	14·5	3·5	
Grade 5	52 (10)	26 (4)	17·7	2·6	
Grade 6	23 (5)	5 (1)	40·7	8·6	0·0002

[a] Based on 1204 subjects with complete information.

[b] Based on 1208 subjects with complete information.

TABLE 5. Relationships of cutaneous microtopography and paraocular photography to history of non-melanotic skin cancer in the Busselton Population Survey, 1981

	History of non-melanotic skin cancer			After control of age in 5-year groups	
	Present	Absent			
Parameter	No. of subjects (%)	No. of subjects (%)	Crude odds ratio	Odds ratio	Significance of trend
Cutaneous microtopography[a]					
Grade 1	0 (—)	24 (2)			
Grade 2	0 (—)	68 (6)	1·0	1·0	
Grade 3	2 (1)	193 (18)			
Grade 4	14 (14)	255 (23)	7·8	3·9	
Grade 5	39 (38)	320 (29)	17·4	3·6	
Grade 6	47 (46)	239 (22)	28·0	9·2	0·004
Paraocular photography[b]					
Grade 1	5 (5)	192 (17)	1·0	1·0	
Grade 2	37 (36)	368 (33)	3·9	1·1	
Grade 3	25 (25)	298 (27)	3·2	0·5	
Grade 4	21 (21)	151 (14)	5·3	0·8	
Grade 5	10 (10)	68 (6)	5·6	1·1	
Grade 6	4 (4)	24 (2)	6·4	1·0	0·528

[a] Based on 1201 subjects with complete information.
[b] Based on 1203 subjects with complete information.

grade 1 microtopograph had a keratosis.) Past history of non-melanotic skin cancer was also significantly, although less strongly, related to microtopograph grade after controlling for the effects of age (Table 5).

The association of solar keratoses with POP was much weaker than with CMT. Nonetheless, the association was still of high statistical significance. After control of age, a subject having grade 6 'crow's feet' was 8·6 times more likely to have a solar keratosis than a subject having grade 1 'crow's feet' (Table 4). No relationship was seen between POP and past history of non-melanotic skin cancer after adjustment for the effects of age (Table 5).

The results of the analysis of inter-observer variation, given in Table 6, showed that the overall proportions of gradings in agreement were 64% for CMT and 60% for POP. When adjusted for chance agreement (kappa) these proportions fell to 54% and 41% respectively, levels quite similar to those described for the assessment of various histopathological features by pathologists (Holman *et al.*, 1982, 1983). Discordant gradings were never greater than one grade apart for CMT or two grades apart for POP. It may be seen from the proportional distributions of gradings in Table 6 that grades 3 to 6 were used most frequently in rating microtopographs, whereas paraocular photographs were more often assessed as grades 1 or 2. Agreement levels tended to be higher on the more commonly assigned grades, even after the higher levels of chance agreement on these grades were taken into account by use of kappa statistics.

TABLE 6. Agreement between two observers on the grading of cutaneous microtopographs and paraocular photographs taken from fifty subjects

Parameter	Proportional distribution of gradings[a]	Proportion in agreement	Kappa statistic	Standard error of kappa
Cutaneous microtopography				
Grade 1	0·03	0·00	−0·03	0·14
Grade 2	0·09	0·44	+0·39	0·14
Grade 3	0·28	0·71	+0·60	0·14
Grade 4	0·15	0·40	+0·29	0·14
Grade 5	0·27	0·74	+0·64	0·14
Grade 6	0·18	0·78	+0·73	0·14
All grades	1·00	0·64	+0·54	0·07
Paraocular photography				
Grade 1	0·37	0·76	+0·61	0·14
Grade 2	0·38	0·58	+0·32	0·14
Grade 3	0·19	0·53	+0·42	0·14
Grade 4	0·02	0·00	−0·02	0·14
Grade 5	0·02	0·00	−0·02	0·14
Grade 6	0·02	0·00	−0·02	0·14
All grades	1·00	0·60	+0·41	0·09

[a] Total number of gradings = 50 × 2 = 100.

DISCUSSION

Previous investigations have provided some results in support of a relationship of skin condition, as assessed by CMT and POP, with past sun exposure. Beagley & Gibson (1980) collected skin moulds of the forearm and dorsum of hand, and questionnaire data on places of residence and past outdoor exposure, from 340 healthy volunteers. They found that microtopograph grade was strongly influenced by age, but after the effects of age were controlled, there was also a highly significant relationship with estimated total lifetime UVR dose (correlation coefficient = 0·68, $P < 0{\cdot}00001$). Daniell (1971) in an analysis based on 901 patients who attended a general medical clinic, determined that smoking, age and outdoor exposure all had significant and independent effects on the production of ‘crow’s feet’. From his results it is possible to calculate that after controlling for age and smoking history, subjects who averaged more than 4 hours of current daily outdoor exposure were 2·5 times more likely to have prominent ‘crow’s feet’ (grades 4 to 6) than persons who spent less than 2 hours per day outdoors ($P < 0{\cdot}00001$).

The demonstration in this study of a strong association, within 5-year age groups, of the presence of solar keratoses and past history of non-melanotic skin cancer with skin changes graded by CMT does not constitute proof that CMT gives a reliable indication of severity of actinic skin damage nor extent of past sun exposure. It is at least consistent, however, with the relationship of microtopograph grade with total lifetime UVR dose observed by Beagley & Gibson (1980). Solar keratoses are generally accepted as having a causal association with sun exposure (Carmichael, 1962; Gordon, Silverstone & Smithurst, 1972; Whiting, 1978), and non-melanotic skin cancers caused by sunlight are thought to result from an accumulation of UVR dose over a prolonged period (Scotto & Fraumeni, 1982; Fears, Scotto & Schneiderman, 1977; Cutchis, 1978; Vitaliano & Urbach, 1980). The results therefore provide substantial indirect evidence for the validity of CMT as an index of total lifetime exposure to the sun. Moreover, it would seem reasonable to conclude that the changes in surface texture graded by CMT reflect, at least in part, the manifestations of actinic skin damage.

The weaker relationship of POP with presence of solar keratoses, and the absence of an association with history of non-melanotic skin cancer, provide little support for the use of POP as an alternative index of actinic skin damage. Furthermore, the degree of inter-observer variation in the grading of paraocular photographs was greater than for cutaneous microtopographs, a factor which may have contributed to the weaker correlation of POP than CMT with risk of solar keratosis and previous skin cancer. Possibly, 'crow's feet' are related to effects of sunlight other than an accumulation of UVR dose over many years. This would explain the weaker (in comparison with CMT), but nonetheless significant association of POP with the presence of solar keratoses, and with current level of outdoor exposure as described by Daniell (1971).

There was in this survey, as in earlier work, evidence of a strong tendency for skin changes graded by CMT (and POP) to worsen with increasing age; this is implied in the effects of control of age seen in Tables 3 and 4. It should not be inferred from this observation that there is necessarily an effect of age on microtopograph appearance which is independent of UVR exposure. The age of an individual probably provides one of the best available estimates of his total accumulated dose of UVR relative to that of other persons in the same population. In controlling for the effects of age, therefore, one is eventually restricting the analysis of variation in actinic skin damage to that due to genetic, geographic and lifestyle factors. If, however, the natural ageing process also contributes to the deterioration of skin surface texture with advancing years, and these changes can be distinguished from those due entirely to sun exposure, then it would be advantageous to refine the CMT grading system so as to exclude the age effects from the assessment of skin damage. A comparative study of CMT in subjects of matched age from two genetically similar populations receiving vastly different levels of UVR exposure (e.g. Australia and the British Isles) might prove useful for this purpose.

Clearly, there is a need for more information relating to CMT, especially with respect to the microanatomical correlates of the skin texture changes graded by the system of Beagley and Gibson and the nature of the linkage of these changes with UVR exposure. Distortion of the microarchitecture of the skin resulting from damage to dermal collagen caused by UV-A (Parrish *et al.*, 1978) is one possible mechanism. There is no reason, however, why a lack of detailed knowledge of the underlying processes should prevent the practical application of CMT as an indicator of severity of actinic skin damage. On the basis of the results of this investigation, we conclude that CMT shows promise as an epidemiological tool, both in studies of the causation of UVR-related diseases, and in the evaluation of measures directed towards their prevention.

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Endothelial cell pathology as a marker for urticarial vasculitis: a light microscopic study

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SUMMARY

Various criteria have been used for the diagnosis of urticarial vasculitis, and for the classification of patients with chronic urticaria. In the present study, semithin Epon-embedded sections were obtained from fifteen patients using a method of fixation optimal for the preservation and examination of the dermal microvasculature. This allowed patients to be classified according to the presence or absence of significant endothelial cell pathology. This feature proved a more reliable indicator of associated humoral abnormalities than the nature of the perivascular infiltrate as seen on paraffin-embedded sections. Endothelial cell necrosis *per se* was a rare event and could not be used as a reliable criterion for the diagnosis of urticarial vasculitis. Most cases showed either endothelial hypertrophy or shrinkage.

A number of different criteria are used for the diagnosis of vasculitis in routine paraffin-embedded sections. Cutaneous varieties which involve postcapillary venules are characterized by four principal features: a neutrophil-rich perivascular infiltrate, red cell extravasation, neutrophil-derived nuclear debris (leukocytoclasia), and deposition of fibrinoid material within the walls of affected vessels. When all these features are present there is little problem in establishing the diagnosis. Difficulties may arise however in situations where one or more of these features is lacking, and these considerations are particularly relevant to the criteria which have been used to diagnose urticarial vasculitis.

The series originally reported by Soter (1977) seemed to form a well-defined clinicopathological entity in that patients were characterized by clinical urticaria, histological vasculitis, elevated erythrocyte sedimentation rates, and evidence of an immune complex-mediated disease process. However, red cell extravasation was not a prominent feature and cases of vasculitis were divided into those with a neutrophil-rich and those with a lymphocyte-rich perivascular infiltrate (Soter *et al.*, 1976). The existence of this latter group is slightly problematic since the only criterion which remains for diagnosing vasculitis on routine sections is the presence of fibrinoid material in blood vessel walls.

In a previous study of urticaria and urticarial vasculitis histopathological criteria were used to divide patients into three groups (Russell Jones *et al.*, 1983a). Group 1 showed a neutrophil-rich perivascular infiltrate with significant leukocytoclasia and evidence of fibrinoid deposition in the walls of cutaneous blood vessels. They therefore fulfilled the criteria for a diagnosis of urticarial vasculitis. Patients without evidence of vasculitis were divided into two further groups. Group 2 patients showed a dense perivascular infiltrate which was predominantly mononuclear but contained significant numbers of neutrophils and/or eosinophils. Leukocytoclasia, if present, was minimal and deposition of fibrinoid material within vessel walls was not seen (though fibrin was present between vessels). Group 3 cases showed a sparse perivascular infiltrate consisting mainly of mononuclear cells with only occasional granulocytes. Immunological investigations in these patients revealed a spectrum of changes with group 2 patients showing features intermediate between the other two groups. Thus circulating immune complexes (CICs) were detected in all group 1 patients, half the group 2 patients but none of the group 3 patients (Russell Jones *et al.*, 1983a). In a similar study by Monroe *et al.* (1981) the corresponding figures for CICs detected by the C1q binding assay were 44, 13 and 0% respectively.

In the present study the presence or absence of significant endothelial cell pathology was used to divide patients into two groups regardless of the predominant cell type in the surrounding infiltrate. The associated immunological findings were then contrasted with those obtained using the three tier classification. In addition electron microscopic studies were used to

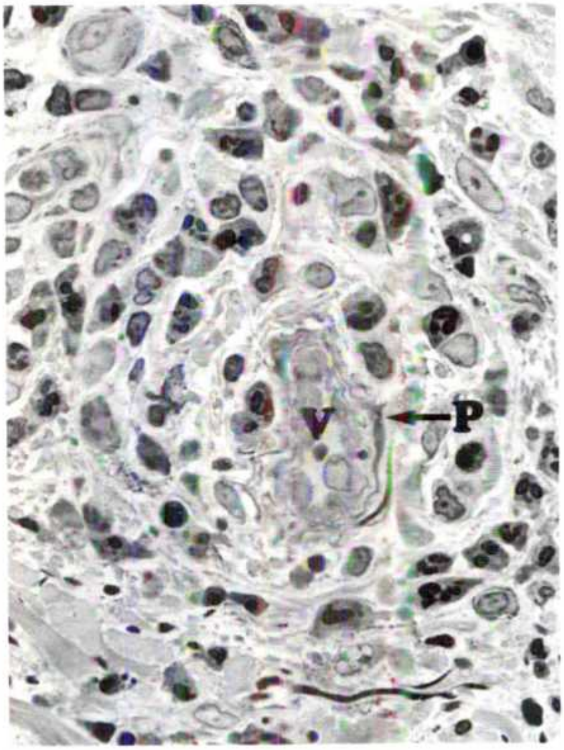

FIGURE 1. A neutrophil-rich infiltrate surrounds a venule (V) characterized by hypertrophied endothelial cells, and surrounded by a continuous layer of pericytes (P). Patient F.B. Forssmann fixative, magnification × 860.

characterize more precisely the endothelial cell changes observed on light microscopy. The ultrastructural observations are to be reported separately.

METHODS

The clinicopathological findings in twenty-four patients presenting with urticarial lesions have been previously documented (Russell Jones *et al.*, 1983a). In the present study biopsies of lesional skin were obtained from fourteen of these patients. One further patient with essential mixed cryoglobulinaemia who presented with urticaria and histological evidence of leukocytoclastic vasculitis was also included in the study. Clinicopathological details of this case have been reported separately (Russell Jones *et al.*, 1983b). In all cases the lesions biopsied had been present for less than 24 h. Semithin and ultrathin sections were obtained using a method which is optimal for the preservation and examination of the human dermal microvasculature (Higgins & Eady, 1981). Briefly, 4-mm punch biopsies were obtained from lesional skin under local anaesthesia (1% lignocaine hydrochloride without adrenaline), divided into three pieces and fixed using one of the following techniques. In three cases the specimens were immersed in half-strength Karnovsky fixative (Karnovsky, 1965) in 0·1 M cacodylate buffer (pH 7·4) with 0·05% $CaCl_2$ for 4 h at room temperature, rinsed in 0·067 M cacodylate buffer and post-fixed in 1·33% osmium tetroxide in S-collidine buffer (pH 7·35) for 2 h on ice. The remaining specimens were immersed in a solution containing 1·5% formaldehyde, 1·5% glutaraldehyde and polyvinylpyrollidone as described by Forssmann *et al.* (1977) at pH 7·35 for 1–3 h on ice, followed by storage in a second fixative similar to the first but with the addition of picric acid and by post-fixation in 1% osmium tetroxide in phosphate buffer (pH 7·4) (Millonig, 1962) for 2 h on ice. After dehydration in ethanol and propylene oxide the tissues were embedded in Epon.

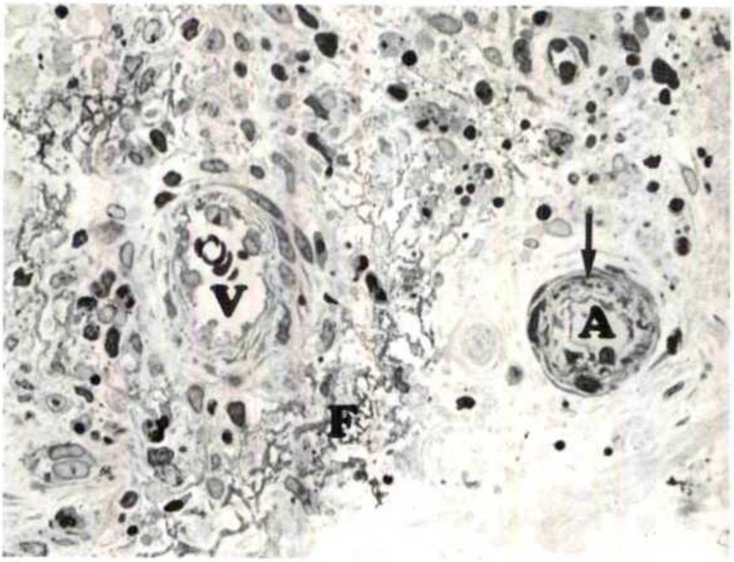

FIGURE 2. A dilated venule (V) is surrounded by heavy deposition of fibrinoid material (F) and leukocytoclasia. An adjacent ascending arteriole (A), identified by the presence of an internal elastic lamina (arrowed) is unaffected by the inflammatory process. Patient S.B. Forssmann fixative, magnification × 540.

Semithin (1 μm) vertical sections stained with methylene blue and basic fuchsin (Huber, Parker & Odland, 1968) were examined by light microscopy.

RESULTS

Endothelial cell changes

Light microscopic examination of 1-μm Epon-embedded sections allows division of the dermal microvasculature into several distinct segments (Higgins & Eady, 1981). Post-capillary venules and venules of the superficial venular plexus are characterized by endothelial cells with round, indented nuclei, an irregular luminal surface and a relatively thick perivascular matrix. In all cases affected vessels were identified as venules of the superficial venular plexus (Fig. 1). In group 1 patients vessels located more deeply within the reticular dermis were affected also. Arterioles, characterized by the presence of an internal elastic lamina, were not involved by the inflammatory process (Fig. 2).

Vessels were examined for the presence of endothelial cell hypertrophy, shrinkage, or necrosis. Hypertrophied endothelial cells were characterized by large pale endothelial cell nuclei and prominent nucleoli (Fig. 3). In three cases this feature was associated with partial or complete luminal obliteration (Fig. 4). However, two of these cases were fixed in half-strength Karnovsky and were associated with endothelial cell blebs (Fig. 5). This change has been previously attributed to fixation in half-strength Karnovsky and was not seen in Forsmann-fixed tissue (Higgins & Eady, 1981). More commonly, affected vessels were dilated and contained either inflammatory cells or red cells (Fig. 6). Endothelial cell shrinkage was observed in some cases (Fig. 7) but this feature was seldom observed in isolation. Other vessels in the same section showed hypertrophy and occasionally contrasting changes were seen in adjacent endothelial cells (Fig. 8). Slight variation in the size and staining properties of endothelial cells

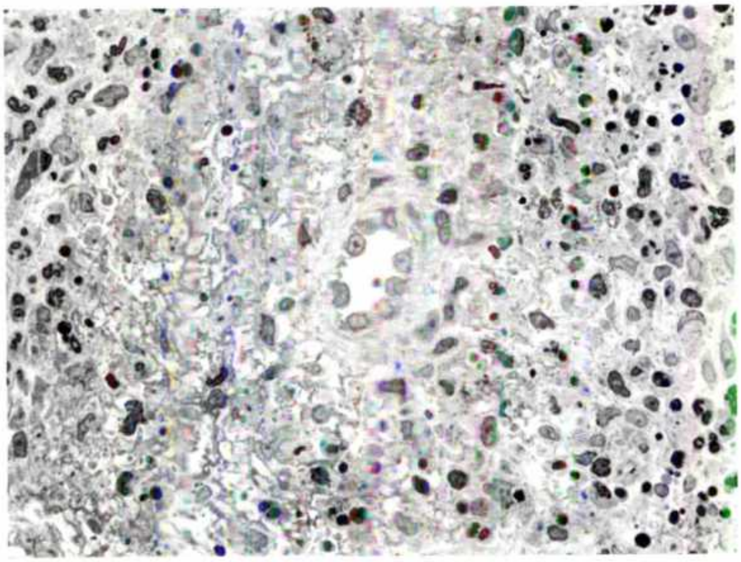

FIGURE 3. Venule shows marked endothelial cell hypertrophy despite the presence of heavy fibrin deposition in the surrounding dermis. Patient S.B. Forssmann fixative, magnification × 540.

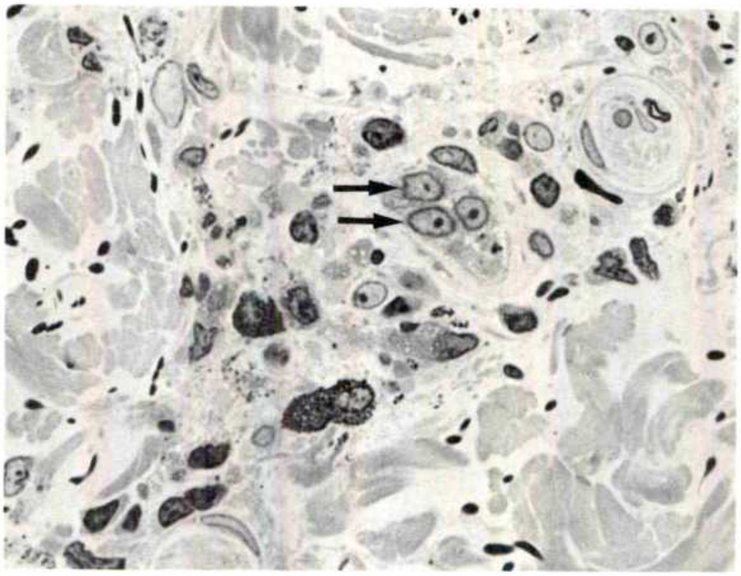

FIGURE 4. Venule shows swollen endothelial cells (arrowed) and partial luminal obliteration. Patient G.R. Half-strength Karnovsky fixative.

may be seen in normal skin. In the present study endothelial changes were therefore graded as mild (+), moderate (+ +) or marked (+ + +).

The presence of endothelial cell necrosis was difficult to identify with certainty by light microscopy. Necrosis implies irreversible damage to the structural integrity of either the nuclear or plasma membranes, features which cannot be identified reliably by light microscopy

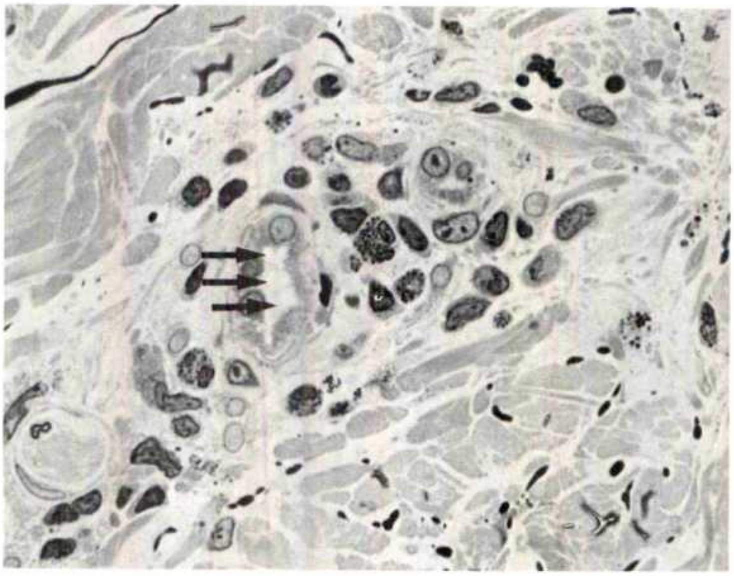

FIGURE 5. Swollen endothelial cells with blebs (arrowed) extend into the lumen of a venule. This probably represents a fixation artefact. Patient G.R. Half-strength Karnovsky fixative, magnification × 720.

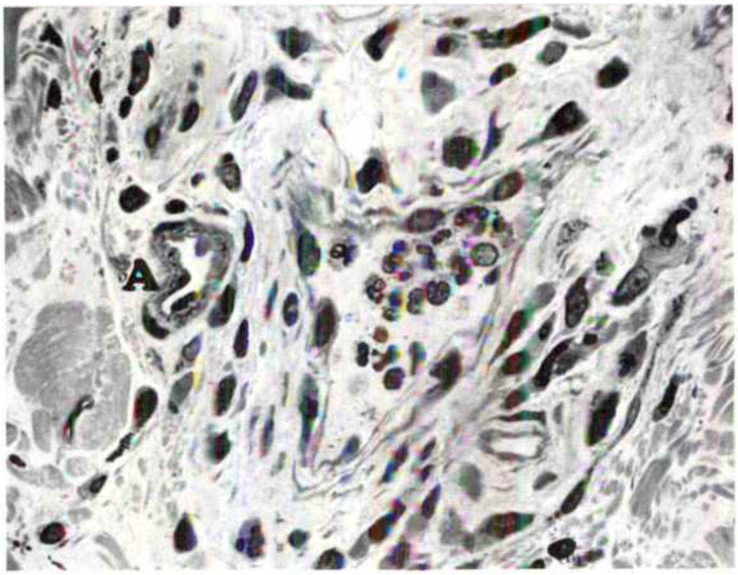

FIGURE 6. Dilated venule contains collections of leukocytes with intraluminal lysis of neutrophils. A = arteriole. Patient L.F. Forssmann fixative, magnification × 690.

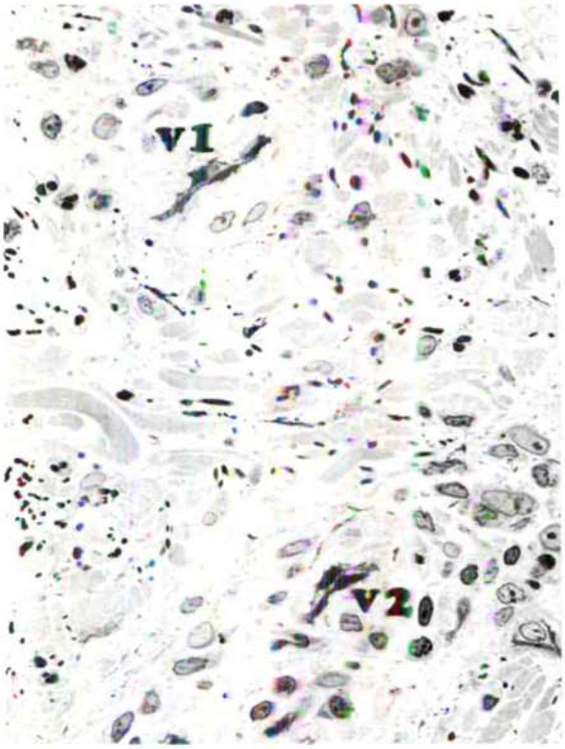

FIGURE 7. Two venules (V1 and V2) show endothelial cell shrinkage and luminal obliteration. Patient D.C. Forssmann fixative, magnification × 540.

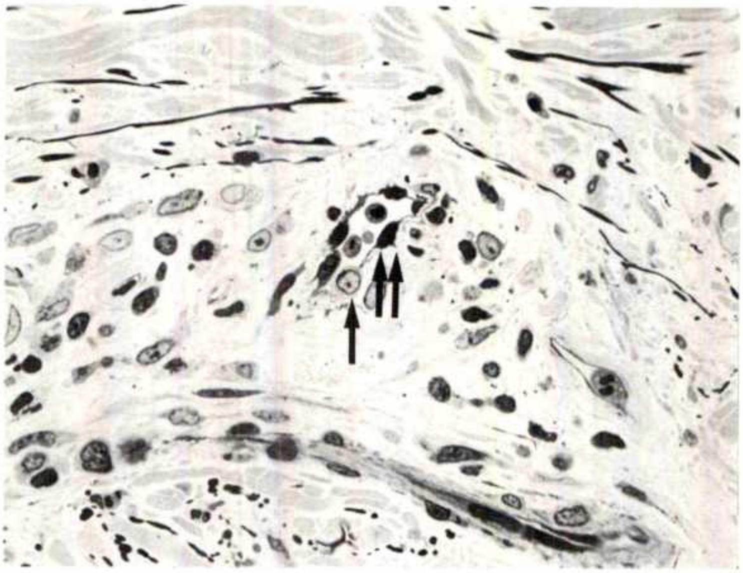

FIGURE 8. Venule shows hypertrophy (single arrow) and shrinkage (double arrow) in adjacent endothelial cells. Patient D.C. Forssmann fixative, magnification × 540.

unless cell lysis has actually occurred. In fact lysed endothelial cells were not seen in this study. Cytoplasmic vacuolization is sometimes regarded as a feature of cell necrosis; this feature was rarely seen (Fig. 9) and could be reliably distinguished from vacuolization of pericytes and other peri-endothelial cells only by electron microscopy.

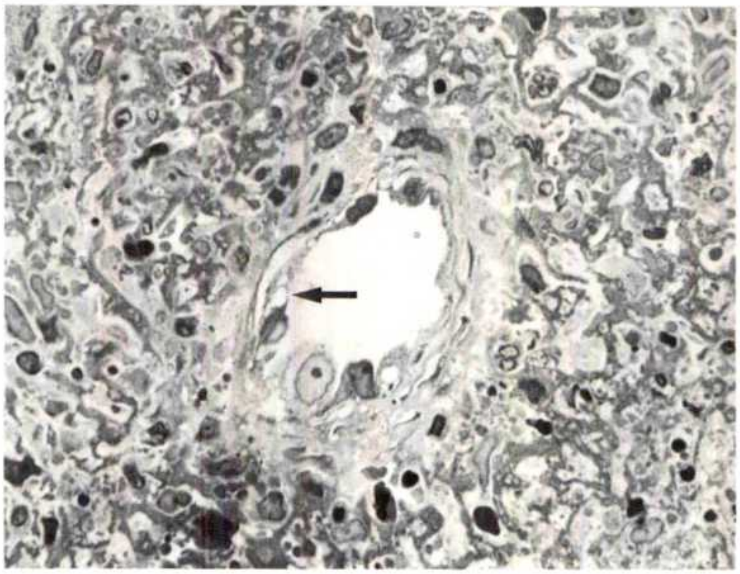

FIGURE 9. Venule shows intracytoplasmic vacuolization (arrowed) of endothelial cells. This was interpreted as evidence of early endothelial cell necrosis. There is massive fibrin deposition and extensive cell lysis in the surrounding dermis. Patient S.B. Forssmann fixative, magnification × 850.

Classification of patients

In our previous study patients were classified into three groups depending upon the nature of the perivascular infiltrate as seen on routine H & E paraffin-embedded sections (Russell Jones *et al.*, 1983a). In the present study patients were divided into groups A and B according to the presence or absence of significant endothelial cell pathology (defined as moderate or marked hypertrophy and/or shrinkage).

Table 1 details the light microscopic findings using semithin sections and compares the two methods of classifying subjects.

TABLE 1. Endothelial cell changes in groups A and B

		Endothelial cell changes			Luminal changes			
Patient	Age/Sex	H	S	N	O	D	Current classification	Previous classification
E.C.	57 M	+	+ +	–	–	+	A	Group 1
S.B.	33 F	+ + +	+ +	+	+	+	A	Group 1
F.B.	61 F	+ + +	+	?	–	+ +	A	Group 1
G.R.*	46 M	+ +	–	?	+	–	A	Group 2
D.C.	32 F	+ +	+ +	–	–	+	A	Group 2
E.K.*	57 M	+ +	+	?	+	–	A	Group 2
L.F.	25 F	–	–	–	–	+ +	B	Group 2
G.C.	32 M	+	–	–	–	+	B	Group 2
R.J.	38 M	+	–	–	–	+	B	Group 2
B.K.*	34 F	+	–	–	–	+	B	Group 2
T.S.	34 M	–	–	–	–	+	B	Group 3
B.G.	27 F	+	–	–	–	–	B	Group 3
C.J.	40 F	–	–	–	–	–	B	Group 3
M.B.	35 F	+	–	–	–	–	B	Group 3
H.J.	38 M	–	–	–	–	–	B	Group 3

* Half-strength Karnovsky.
H = hypertrophy.
S = shrinkage.
N = necrosis.
O = obliteration.
D = dilatation.

TABLE 2. Complement levels in group A

Patient	CH_{50}	C4	C3	C3CP	Comment
E.C.	↓	↓	N	No	Cryoglobulinaemia
S.B.	↓	↓	↓	Yes	Hypocomplementaemia
F.B.	N	N	N	No	Normocomplementaemia
G.R.	N	N	N	No	Sample obtained during disease remission
D.C.	N	N	N	Yes	Evidence of C3 conversion
E.K.	N	N	N	No	Normocomplementaemia

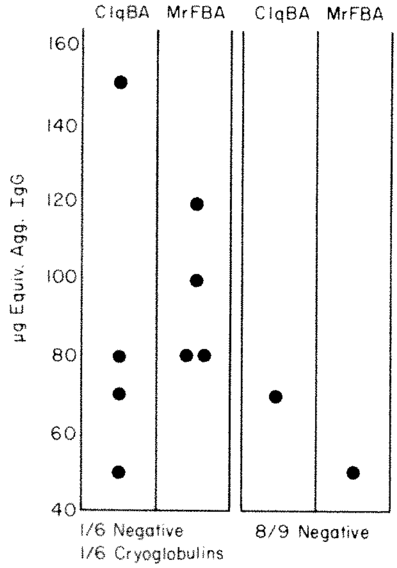

FIGURE 10. Levels of circulating immune complexes in groups A and B.

Table 2 details the complement levels in group A patients. One patient showed hypocomplementaemia with low levels of CH50, C4 and C3. The case of cryoglobulinaemic vasculitis showed low levels of C4 without evidence of C3 conversion and one further case had evidence of C3 conversion without hypocomplementaemia. Thus three of six patients had complement abnormalities. No complement abnormalities were found in group B patients.

In our previous study circulating immune complexes (CICs) were detected by the C1q binding assay (C1qBA) and the monoclonal rheumatoid factor binding assay (MrFBA) (Russell Jones *et al.*, 1983a). The results showed that all of group 1 patients and half of group 2 patients but none of the group 3 patients had evidence of CICs. Figure 10 documents the equivalent results in groups A and B. All but one of the group A patients had raised levels of CICs; the one exception was a group 2 patient whose serum sample was obtained during a period of disease remission. By contrast all but one of the group B patients had normal levels of CICs; the exception was again a group 2 patient with a minor degree of endothelial cell hypertrophy. Serum cryoglobulins were of some value in distinguishing group A and B. Apart from the patient with mixed cryoglobulinaemia two out of five group A patients had elevated levels. This compares with only one out of nine in group B.

DISCUSSION

The morphological changes identified in this study raise important conceptual issues in relation to urticaria and urticarial vasculitis. First it is apparent that the presence of endothelial cell necrosis is an unusual finding and cannot be used as a reliable method of defining patients with urticarial vasculitis. Only one patient in the present study showed definite evidence of endothelial cell necrosis and this was associated with massive fibrin deposition and leukocytoclasia in the surrounding dermis. By contrast five other patients in group A showed either endothelial cell hypertrophy or shrinkage without evidence of irreversible endothelial cell changes. For these reasons the term leukocytoclastic vasculitis should be used in preference to necrotising vasculitis, at least in this particular variety of cutaneous vasculitis. Similarly the term venulitis should be used in preference to angiitis, since in all cases the affected vessels were identified as venules of the superficial venular plexus.

A second aspect of this study concerns the method of fixation. The modified Forssmann technique is ideal for the preservation and examination of the dermal microvasculature (Higgins & Eady, 1981). By contrast, tissue fixed in half-strength Karnovsky showed cytoplasmic blebbing and other endothelial cell changes relating to the fixation method. It is quite possible that previous studies have not always distinguished between this type of artefactual change and true endothelial cell necrosis (compare Figs 5 and 9).

A third aspect of this study concerns the associated immunopathological features. Evidence of complement changes and raised levels of circulating immune complexes were virtually confined to group A patients. Group B patients showed no complement abnormalities and only one patient had evidence of raised CICs. In previous studies of urticaria, patients have usually been classified on the basis of the perivascular infiltrate using paraffin-embedded tissue (Monroe *et al.*, 1981). In our own previous study we employed a three tier classification and demonstrated the existence of a group of patients showing histopathological and immunopathological features intermediate between vasculitis and ordinary urticaria (Russell Jones *et al.*, 1983a). In our present study patients in group A included all of the group 1 cases and some of the group 2 cases, whilst group B included all the group 3 cases and the remainder of the group 2 cases. These findings indicate that the presence or absence of significant endothelial cell changes is in some way a more reliable predictor of associated humoral abnormalities than the nature of the perivascular infiltrate.

Finally, it is necessary to consider the most appropriate method of defining vasculitis in patients who present with urticaria. Clearly histopathological criteria are paramount, but the actual criteria used are the subject of some disagreement. Phanuphak, for example, defined vasculitis in terms of a cellular infiltrate within the walls of cutaneous blood vessels (Phanuphak *et al.*, 1980). This feature was seen in 52% of cases, but the vasculitis group, so defined, did not differ from the non-vasculitis group in terms of arthralgia, elevated ESR, or hypocomplementaemia. Clearly this group of patients is very different from those described by Soter (1977). From our own studies, it would be quite legitimate to restrict the definition of vasculitis so that only those cases showing fibrinoid change and leukocytoclasia were included (group 1). Equally, however, the definition could be extended to include both groups 1 and 2, in which case the number of patients will increase but a proportion will lack CICs or other evidence of humorally mediated mechanisms. Alternatively semithin sections and the presence of significant endothelial cell pathology provides a third method of classifying urticaria in which vasculitis is equated with patients in group A. This demonstrates that definitions of vasculitis depend upon the interests and facilities available to different investigators who may arbitrarily extend their diagnostic criteria in order to include larger or smaller numbers of patients. From our own point of view we would argue that different methods are valid provided that the criteria used are clearly stated and provided that they have some relevance either to prognosis or to management. Our own study has shown that the degree of endothelial cell hypertrophy and/or shrinkage is a useful criterion for distinguishing immune complex-mediated urticaria from cases where evidence of CICs is not found. However, this classification requires the use of semithin Epon-embedded sections, a facility which is not routinely available in all departments. For routine histopathology therefore, fibrinoid change and leukocytoclasia remain the most reliable and reproducible criteria for distinguishing patients with vasculitis from those with ordinary urticaria.

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Mechanisms of pustule formation in cutaneous bacterial and viral infections: alterations of neutrophil membrane surface receptors

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SUMMARY

Changes in membrane surface receptors have been demonstrated by rosetting methods in polymorphonuclear leukocytes obtained from the pustules of different infectious dermatoses. There was a distinct difference in numbers of receptors in neutrophils from bacterial and viral pustules, i.e., C3b receptors were decreased on neutrophils from both bacterial and viral pustules, whereas IgG-Fc receptors were decreased only in neutrophils from viral pustules. The difference appears to be due to variations in the defence mechanisms against the invading micro-organisms.

Polymorphonuclear leukocytes (PMNs) play an active role in the mediation of inflammatory responses. It has been established that these responses occur as a co-ordinated series of events which include adherence to endothelium, extravascular migration, directed migration (chemotaxis), membrane recognition, phagocytosis, fusion of lysosomes with phagocytic vacuoles, and a burst of oxidative metabolism (Goldstein, 1980). In various kinds of infectious dermatoses, pustules are produced as a result of these inflammatory reactions.

It has been assumed that PMNs recognize the presence of invading micro-organisms through two major membrane receptors, i.e., receptors for the Fc portion of IgG and those for fragments of C3. In order to elucidate the mechanisms involved in inflammatory processes taking place in these disorders, we examined the changes in the membrane surface receptors of PMNs from pustules of bacterial and viral infections, using rosette formation assays with sensitized ox red blood cells.

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METHODS

Collection of PMNs

These were obtained from pustules caused by bacterial infections; nine lesions of folliculitis and seven of pustular acneform eruptions. The cells from pustules of viral origin were from eight patients with herpes zoster lesions. PMNs were collected by puncturing the pustules after disinfection of the skin surface with 70% ethyl alcohol.

Control PMNs were obtained from nine normal donors by sedimentation of heparinized blood in 6% dextran, followed by Ficoll-Hypaque separation (Boyum, 1968). Each leukocyte preparation consisted of more than 85% PMNs.

EA (IgG) and EA (IgM) C rosetting assay

PMNs with IgG-Fc receptors and C3b receptors form EA and EAC rosettes, respectively. The microplate method (Tachibana & Ishikawa, 1973) was used. In brief, approximately 1×10^4 PMNs in 1 μl Hanks' balanced salt solution, and 1 μl of fetal calf serum were put into the well of a microplate (JIMRO, Japan). IgG-coated ox red blood cells (EA) or IgM-C3-coated ox red blood cells (EAC) (JIMRO, Japan), 2×10^6 cells in 10 μl of gelatin veronal buffer, were added. After incubation at room temperature for 30 min, the plate was turned over and left for another 30 min. Then the plate was washed gently with phosphate-buffered saline to remove unattached cells. PMNs in the well were stained for examination of rosettes. Leukocytes forming rosettes with three or more red blood cells were considered positive. PMNs were simultaneously checked for viability by Trypan Blue dye exclusion. Only those

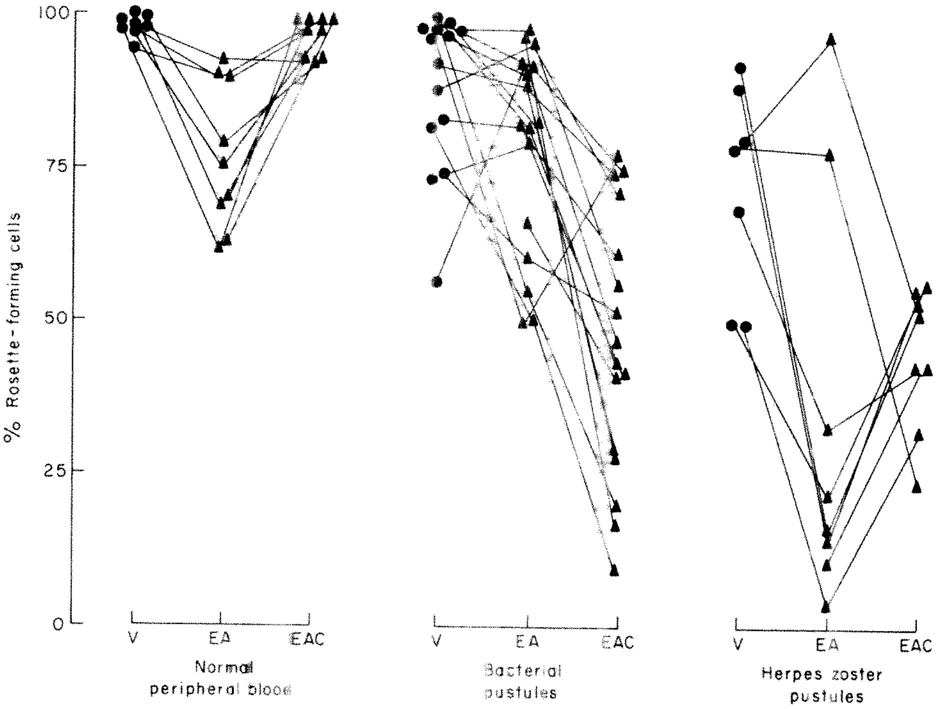

FIGURE 1. Rosette formation rates of PMNs for EA and EAC, with viability (V).

preparations showing more than 90% viable cells in peripheral blood and showing more than 50% viable cells in pustules were used. Statistical significance was determined by Student's *t*-test.

RESULTS

The percentages of EA and EAC rosettes are shown in Fig. 1. PMNs from the peripheral blood of normal donors formed 76·4 ± 11·3% EA rosettes, and 96·4 ± 3·2% EAC rosettes. There was no correlation between the percentages of formation of rosettes with the maturity of PMNs observed in the peripheral blood.

When compared with the results of tests on PMNs from peripheral blood of normal donors, the PMNs from bacterial pustules showed a decrease in formation of EAC rosettes, these being only 46·2 ± 21% ($P < 0{\cdot}01$). There was no difference in the formation of EA rosettes 78·3 ± 16.3%. Thus there was a decrease in detectable C3b receptors but not of IgG-Fc receptors.

Formation of both EA rosettes, 17·0 ± 9·1% ($P < 0{\cdot}01$) and of EAC rosettes 44·0 ± 11·4% ($P < 0{\cdot}01$) was decreased in PMNs from pustules of herpes zoster. These percentages omit the results of two exceptional cases who showed greater numbers of rosetting cells than the cells of the remainder of patients with herpes zoster. There was therefore a decrease in cases of viral infection, compared with the PMNs of peripheral blood from normal donors.

DISCUSSION

It has been estimated that 75–90% of human peripheral blood PMNs bear receptors for IgG-coated particles and over 90% bear receptors for fragments of C3 on their surfaces (Goldstein, 1980). In our results 76·4 ± 11·3% of peripheral blood PMNs formed EA rosettes (bearing receptors for IgG-Fc), and 96·4 ± 3.2% formed EAC rosettes (bearing receptors for C3b).

We found that there is a marked decrease in C3b receptors on PMNs from the pustules of bacterial infections. Sakurada *et al.* (1981) also found such a decrease in C3b receptors on PMNs obtained from abscesses of the oral cavity. These facts indicate that the receptors for C3b have already been occupied by complement binding particles, while the receptors for IgG-Fc remain unoccupied and suggest that PMNs in the pustules of bacterial infections recognize bacteria chiefly through complement activation on their surfaces (Pillemer *et al.*, 1955) rather than through specific IgG antibodies.

In contrast, significant decreases in both IgG-Fc and C3b receptors were found on PMNs from the pustules of herpes zoster. Thus the receptors for both IgG-Fc and C3b have already been occupied by IgG binding particles and complement binding particles respectively, suggesting that PMNs in the pustules of herpes zoster recognize virus antigens through both of their IgG-Fc and C3b receptors.

These findings provide indirect evidence that PMNs in the pustules of bacterial and viral dermatoses recognize the invading micro-organisms in different manners, i.e. in bacterial infections, complement receptors on the surface of PMNs seem to play a major role in recognizing the bacterial antigens, while in viral infections both IgG-Fc and complement receptors are likely to play a major role probably through IgG and complement fixing antibodies.

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Alopecia areata: light and electron microscopic pathology of the regrowing white hair

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SUMMARY

Light and electron microscopic studies have been carried out on fifteen regrowing white anagen hair follicles from seven patients with alopecia areata. Seven bulbs showed evidence of cell injury that was concentrated in cortical keratocytes. Lower bulb matrix cells appeared undamaged though in six bulbs apoptotic degeneration was seen in the lower bulb keratocytes, perhaps indicating early catagen transformation. Melanocytes were identified in all the bulbs except those from one patient. The number of melanocytes and their melanization were much less than in the normal pigmented follicle, and pigment transfer was rarely seen. It is proposed that alopecia areata is a disease of differentiating cortical keratocytes. The failure of pigmentation in the regrowing white hair may be post-inflammatory, but the various other pigmentary features of alopecia areata, especially the sparing of senile white hairs, suggest that pigmentary mechanisms in the hair bulb are of primary importance in the pathogenesis of this disorder.

Alopecia areata exhibits a variety of pigmentary features. These include an association with vitiligo (Muller & Winkelmann, 1963) particularly in the Vogt-Koyanagi-Harada syndrome where up to 70% of cases have hair loss (Rosen, 1945), peri-naevoid alopecia (Yesudian & Thambiah, 1976), and there may be pigmentary changes in the eyes (Brown, Pollard & Jarrett, 1982). When regrowth occurs the hair is often initially non-pigmented. Perhaps the most significant observation is that senile white hairs are less susceptible to the disease. Some individuals with alopecia areata show complete sparing of senile white hair although in others this is a relative phenomenon and in a few patients white hairs and pigmented hairs are equally involved.

Our knowledge of the pathophysiology of senile greying is limited. Dopa reactivity (Bloch, 1921) and tyrosinase activity (Fitzpatrick, Brunet & Kukita, 1958) are absent in senile white hair bulbs and reduced in grey hairs. Functional melanocytes are not found in the majority of senile white hair bulbs (Fitzpatrick, Szabo & Mitchell, 1965; Herzberg & Gusek, 1970; Sato, Kukita & Jimbow, 1973) though Sato *et al.* (1973) reported the presence of indeterminate cells which they felt were effete melanocytes. The mechanism of this decline in melanocyte activity is unknown

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but may be more complex than the simple 'wearing out' of melanocytes. We propose several possibilities for the sparing of white hairs in alopecia areata.

(1) Although similarly attacked, white hair is more resistant to the disease than pigmented hair.
(2) White hair follicles spend longer in telogen than pigmented hair follicles.
(3) The disease target is reduced or absent in white hairs.

To determine the influence of alopecia areata on melanin pigmentation in the hair bulb we have studied the pathology of the regrowing white hair. If white hairs are spared because some aspect of the pigmentary mechanism forms the disease target, the failure of pigmentation in the regrowing hair may be a primary event in the pathogenesis rather than being merely post-inflammatory.

METHODS

Scalp biopsies were taken from areas showing regrowth of white hair in seven patients with alopecia areata. In all seven the pre-morbid hair was fully pigmented. Individual anagen follicles were removed under a dissecting microscope within a few minutes of biopsy and fixed in phosphate-buffered 3% glutaraldehyde. After post-fixing in osmium tetroxide and dehydration in an ascending ethanol series the specimens were embedded in Araldite. The bulbs were trimmed longitudinally until the dermal papilla was reached and then 1 μm and ultrathin sections were cut at three or four levels to include the apex of the papilla. One micron semithin sections were stained with toluidine blue and examined by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400 electron microscope.

TABLE 1. Ultrastructural pathology of hair bulbs

Case	Age	Sex	Hair bulb	Melanocyte numbers	Tissue injury	Mononuclear cell infiltration	Apoptosis
1	55	F	i	0	0	0	0
			ii	0	0	0	0
			iii	0	0	0	0
			iv	0	0	+	0
2	48	F	i	+	0	0	0
			ii	+	+	+	+
3	31	M	i	+	0	0	0
4	33	M	i	+	+ +	+	0
			ii	+	+ +	+	+
			iii	+	+	+	+
5	31	M	i	+ +	0	+	+
6	40	M	i	+ +	+ +	+ +	0
7	39	M	i	+ +	0	+	0
			ii	+ +	+ +	+ +	+
			iii	+ +	+ +	+ +	+

Figures refer to numbers identified in a single section through a complete hair bulb.
Melanocytes: $+ < 2$, $+ + \geqslant 2$. Mononuclear cells: $+ < 6$, $+ + \geqslant 6$.

RESULTS

Fifteen regrowing white anagen hair follicles from seven patients were studied. Patient details and the pathological findings are summarized in Table 1.

Light microscopy

Five follicles showed pathological changes in the presumptive cortex in the upper part of the hair bulb. Cortical cells showed loss of their normal longitudinal orientation and swelling and vacuolation of cytoplasm. Around the upper pole of the dermal papilla there was deposition of amorphous staining material within the epithelium (Fig. 1). Matrix cells in the lower bulb appeared normal in all fifteen follicles and mitoses were always present. The root sheaths also looked normal. The cellularity of the dermal papilla relative to its volume varied considerably but this did not correlate with the degree of tissue damage. There was a peribulbar inflammatory mononuclear cell infiltrate around two follicles.

Electron microscopy

Seven of the fifteen follicles showed evidence of non-specific cell injury in cortical keratocytes in the upper part of the hair bulb. These changes included cytoplasmic swelling and vacuolation, pouch-like protrusions of cytoplasm and lysis of plasma membranes. In five bulbs there were areas of frank cellular necrosis. This was seen throughout the cortex but was most marked around the upper pole of the dermal papilla where there were large deposits of granular necrotic debris within the epithelium (Figs 2 and 3). This was the amorphous-staining material seen at

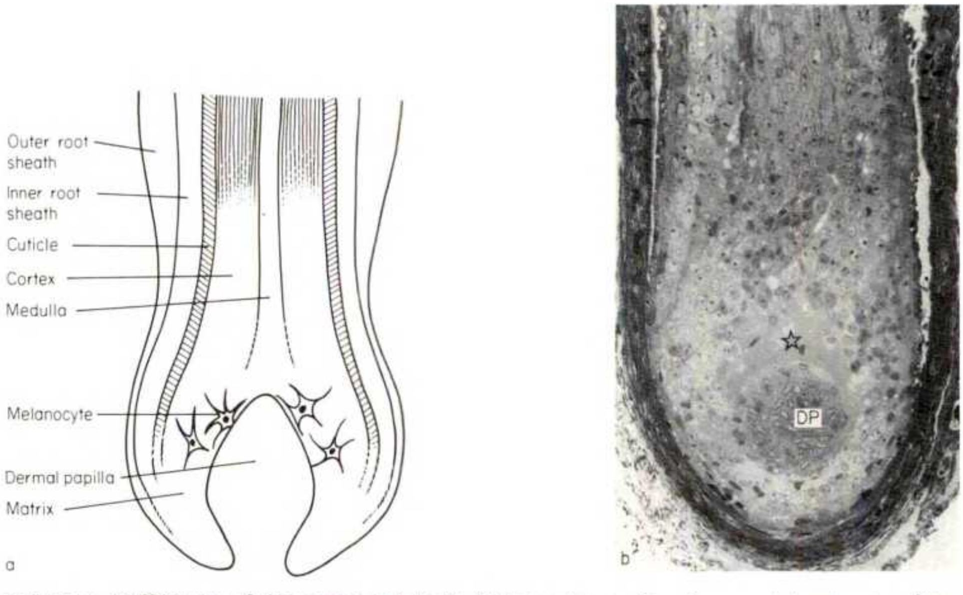

FIGURE 1. (a) Diagram of the anagen hair bulb. Melanocytes are found amongst keratocytes of the presumptive cortex, i.e. cells differentiating from matrix and destined to form hair shaft cortex. (b) Alopecia areata. Regrowing white hair. Keratocytes in the presumptive cortex show loss of normal orientation, cytoplasmic swelling and vacuolation. There is a large area of amorphous staining material (star) around the upper pole of the dermal papilla (DP). (× 190.)

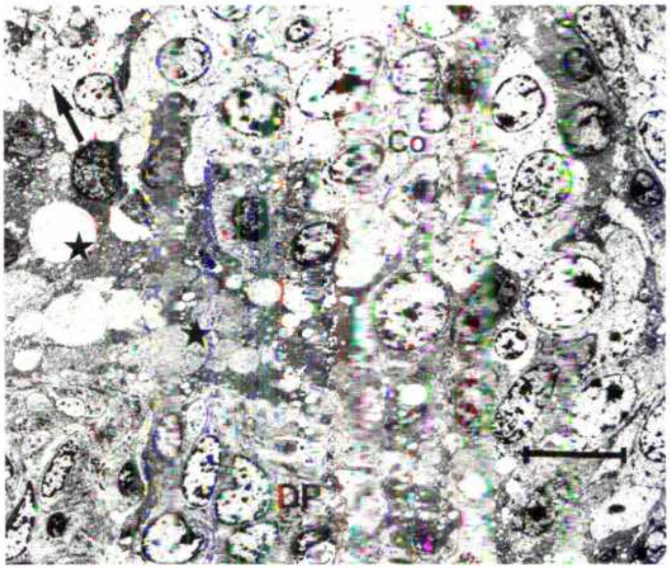

FIGURE 2. Upper bulb. Keratocytes showing degenerative changes are seen throughout the presumptive cortex (Co). Around the dermal papilla (DP) there are large deposits of necrotic debris (stars). Arrow indicates direction of hair growth. Bar = 10 μm.

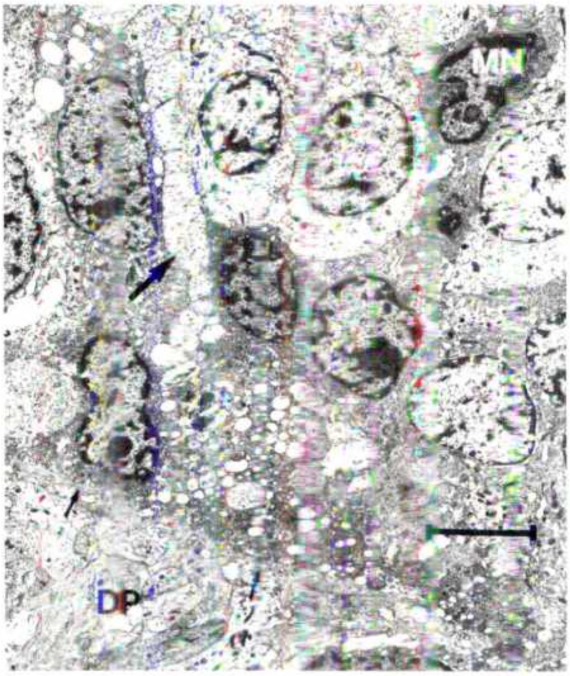

FIGURE 3. Upper bulb. Cortical keratocytes show features of cell injury including vacuolation of cytoplasm, lysis of plasma membranes and cytoplasmic protusion (large arrow). Necrotic debris is seen above the basement membrane (small arrows). DP = dermal papilla. MN = mononuclear cell. Bar = 5 μm.

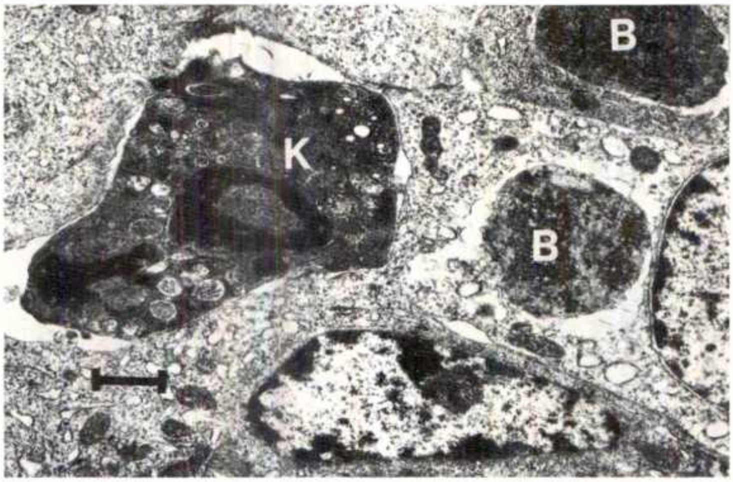

FIGURE 4. Lower bulb matrix. K = apoptotic keratocyte. B = apoptotic bodies. Bar = 1 μm.

light microscopy. The central location of these changes suggested that medullary cells were also involved but cells containing medullary granules were not seen in the damaged tissue. Medullary granules are not always evident at this level in the follicle and medullary cells normally show prominent cytoplasmic vacuolation. However, in two follicles the only indications of cell injury were protrusions of cytoplasm in a few cortical keratocytes. In both of these follicles the medullary cells looked normal. Although fixation artefact can simulate some of these changes they were not seen in other parts of the hair bulb nor in non-keratocytes within the cortex. Matrix cells in the lower bulb did not show evidence of non-specific injury and mitotic activity was present in all the specimens. In six bulbs, however, apoptotic degeneration of individual keratocytes was seen in the lower half of the bulb. Apoptotic bodies were also observed in adjacent keratocytes (Fig. 4). Apoptosis was not seen in the upper bulb.

Melanocytes were found in all the bulbs except those from case 1. In cases 2, 3 and 4 melanocytes were present but were few in number and were only revealed with serial sectioning. Melanocytes were more numerous in the bulbs from cases 5, 6 and 7 but did not reach the population density of normal pigmented follicles. Dendritic processes were poorly developed and pigmentary activity, in terms of melanosome numbers and melanization, was much less than in normal hair bulb melanocytes. Melanin deposition in the melanosomes was nevertheless always apparent. Occasionally there was autophagocytosis of melanosomes within the melanocyte. Melanosomes were seldom observed within keratocytes. Some melanocytes contained a few lipid vacuoles (Fig. 5) but they did not show the degenerative changes seen in cortical keratocytes unless there was necrosis of adjacent keratocytes.

Infiltration of the bulb by inflammatory mononuclear cells, both lymphocytes and histiocytes, was found in the majority of specimens. This was present throughout the epithelium and in the dermal papilla but was concentrated in areas of tissue damage. Melanophages were invariably present in the dermal papilla and pigment-laden macrophages were sometimes found within the epithelium. Mast cells were found in the papillae of two bulbs. There was reasonably close correlation between the density of the inflammatory infiltrate and the degree of tissue damage. In five bulbs no inflammatory cells were found.

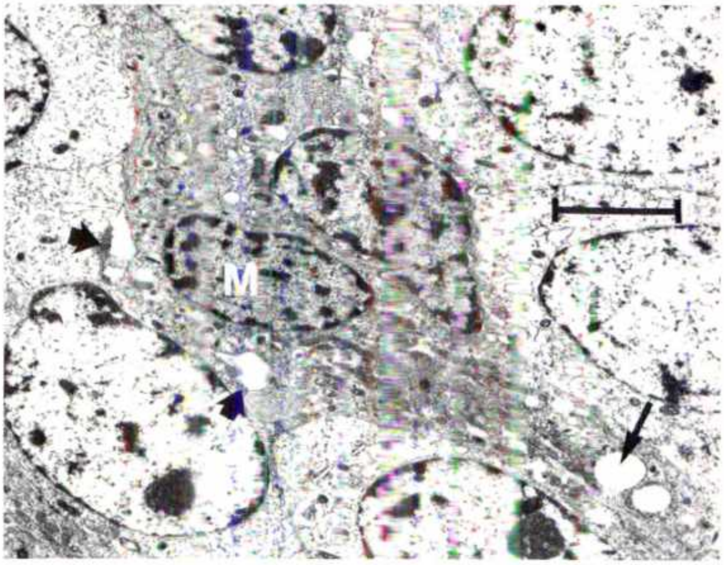

FIGURE 5. Upper bulb. Melanocyte (M) showing two lipid vacuoles in cytoplasm (thin arrow). Melanosomes are few and rather poorly melanized and there is no evidence of pigment transfer. Adjacent keratocytes show dilution of cytoplasmic and nuclear material to varying degrees and degenerative changes in organelles. These changes are not seen in the melanocyte. There are small intercellular deposits of granular debris (fat arrows). Bar = 3 μm.

DISCUSSION

The fifteen regrowing white hairs of alopecia areata that we studied all showed impairment of pigmentary activity, but there was no single site at which pigmentation was uniformly interrupted. Melanocytes were fewer in number and melanization was generally much less than in the normal hair bulb melanocyte. The paucity of melanosomes within keratocytes and the autophagocytosis of melanosomes within melanocytes also suggest defective pigment transfer. There was no evidence of melanocyte destruction except when adjacent keratocytes were severely damaged. On the other hand, these pigmentary changes were present even when there was no evidence of structural damage to keratocytes and where there was little or no inflammatory cell infiltration.

In a preliminary report of this work we stated that keratocytes were undamaged in the regrowing white hair (Messenger, Cawood & Bleehen, 1982). Further experience has shown that this is not always the case and we were able to find evidence of injury to cortical keratocytes in seven of the fifteen bulbs examined. The type of cell injury seen in cortical keratocytes is not specific and could be caused by a variety of disease processes such as ischaemia, toxin damage or immune-mediated cytotoxicity. However, the restriction of cell injury to the cortex and its absence in other parts of the follicle suggest that the cortical keratocyte is the primary disease target within the hair follicle. The infiltration of the hair bulb by inflammatory mononuclear cells lends support to the hypothesis that alopecia areata is mediated by an immune mechanism, although their absence in six bulbs may indicate that their role is not necessarily primary. Lower bulb matrix cells did not show the degenerative changes of cell injury in any of the specimens examined. The apoptotic degeneration seen in lower bulb keratocytes in six follicles may indicate impending catagen (Weedon & Strutton, 1981).

Changes in the dermal papilla have been noted previously in alopecia areata. Van Scott & Ekel (1958) described an increase in the ratio of papillary to matrix cells in anagen follicles in alopecia areata. Some of the follicles that we examined also showed this change which was at least partly due to the presence of inflammatory cells. However, in the majority of bulbs the cellularity of the papilla and matrix appeared normal even when tissue damage was present.

We propose, therefore, that alopecia areata is a disease of the differentiating cortical keratocyte. On the basis of this hypothesis we can explain a number of the clinical and pathological features of alopecia areata. An attack directed against the differentiating cortical keratocyte in the anagen follicle results in a focal disruption of keratinization and the hair shaft fractures at this point on reaching the skin surface. The follicle is unable to continue in anagen and goes into catagen. The process of de-differentiation through catagen into telogen takes place reasonably normally and a club is produced (Eckert, Church & Ebling, 1968). The continued production of hair shaft during this period of involution indicates that matrix activity is not abruptly halted and also that cortical cells retain the ability to produce keratin. Whilst in telogen the follicle is probably safe from attack and this may explain the non-destructive nature of the disease. Anagen recommences as matrix activity is resumed but anagen development is halted at a stage equivalent to anagen III/IV (Van Scott, 1958). It is at this stage of anagen that cortical cells begin to differentiate (Chase, Rauch & Smith, 1951).

The pigmentary disturbance in the regrowing white hair is most easily explained as representing partial or incomplete melanocyte activation in early anagen. This may simply be a toxic response to local inflammation although there was no correlation between impairment of pigmentation and the degree of inflammatory cell infiltration. Alternatively, an attack directed at cortical keratocytes may secondarily impair their ability to accept pigment and perhaps to initiate melanocyte activity. A third possibility is that the attack is specifically directed against mechanisms in the cortical keratocyte which are responsible for regulating pigmentation. It is generally believed that the epidermal keratocyte is actively involved in the control of pigmentation. In the hair follicle the symbiotic relationship between cortical keratocyte and hair bulb melanocyte is particularly striking. 'Dormant' melanocytes are present in small numbers in the telogen bulb (Sugiyama & Kukita, 1976). Functional activation of melanocytes occurs in anagen III and pigment is transferred to cortical keratocytes (Kukita, 1957). Intense pigmentary activity continues throughout anagen until the approach of catagen. Cessation of pigmentation pre-dates other indications of catagen involution resulting in the proximal white tip of the telogen club hair (Montagna & Parakkal, 1974). Thus melanocyte activity closely parallels cortical differentiation and may even be dependent on it. The anatomical position of functional melanocytes in the upper half of the anagen bulb and their near absence in the lower bulb matrix also indicate that cortical differentiation is necessary for pigment transfer to take place. It is possible that senile greying occurs, not because melanocytes wear out, but because of a decline in the ability of cortical cells to initiate melanocyte activity. If the disease attack in alopecia areata is indeed directed against this putative pigmentary mechanism on the keratocyte this would then explain the sparing of senile white hairs. Indirect support for this hypothesis comes from a recent study of vitiligo in which degenerative changes, similar to those seen in cortical cells in alopecia areata, were described in the basal keratocytes in non-lesional skin (Moellmann *et al.*, 1982). The authors ascribed this to melanocytotoxicity but an intriguing feature was that the melanocytes appeared undamaged.

Finally, it has been suggested that alopecia areata is not a single clinico-pathological entity because of the variability in clinical patterns and disease associations (Ikeda, 1965). The sparing of senile white hair is also a variable feature. The proposal that alopecia areata is a disease of differentiating cortical keratocytes would encompass this concept as one would expect the hair follicle to follow a similar reaction pattern whatever the precise nature of the disease target.

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Immunological profiles in alopecia areata

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SUMMARY

Cell-mediated immunity and auto-immune phenomena were investigated in sixty patients with active alopecia areata of various degrees of severity. Serum auto-antibodies to thyroid antigens were detected in twenty-three patients. Examination of T-lymphocyte populations, lymphocyte DNA synthesis, and lymphokine production in response to mitogen stimulation revealed no differences between the sixty patients and matched healthy control subjects. However, patients with thyroid auto-immunity and/or the presence of alopecia totalis or universalis showed significant reductions in interactive T lymphocytes (recognized by rosette formation with human B lymphoblastoid cells) and diminished production of leukocyte migration inhibition factor in response to stimulation with phytohaemagglutinin. This suggests that immune mechanisms may be involved in the pathogenesis of alopecia areata which is associated with thyroid auto-immunity or which progresses to total hair loss.

Previous reports have presented conflicting data on cell-mediated immunity and auto-immune phenomena in patients with alopecia areata. For example, some studies have revealed diminished numbers of circulating T lymphocytes (Brown, Olkowski & McLaren, 1977; Gianetti *et al.*, 1978; Friedmann, 1981a) or a significant association with auto-immune thyroid disease (Cunliffe *et al.*, 1969; Friedmann, 1981b), but others have failed to confirm these findings (Herzer *et al.*, 1979; Gu *et al.*, 1981; Filipovitch *et al.*, 1982; Muller & Winkelmann, 1963; Main *et al.*, 1975). Many of these studies involved only small numbers of patients. The present investigation was undertaken in an attempt to resolve these discrepancies by evaluating cell-mediated immunity and auto-immune phenomena in sixty patients with alopecia areata of various degrees of severity.

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METHODS

Sixty patients were investigated, including eighteen males (ages 11–68 years, mean 27·5, s.e. 4·1) and forty-two females (ages 7–58 years, mean 27·7, s.e. 1·9). Thirty-two patients (eight males, twenty-four females) had alopecia areata limited to circumscribed areas of the scalp and all these had signs of active disease. Twenty-eight patients (ten males, eighteen females) had alopecia totalis or universalis, and were considered as one group. None of these patients had received any form of therapy for alopecia within the 3 months preceding investigation, and none displayed clinical evidence of endocrine or auto-immune disease. Patients in whom auto-antibodies to thyroid antigens were subsequently detected were further investigated for thyroid function which was found to be normal in all such patients.

The control population consisted of normal, healthy subjects matched for sex and, where possible, age. The exceptions to the latter were the controls for the six children in the study who were aged 7–12 years. Blood samples were obtained from patient and control subjects after fully informed consent. Samples were coded and tests read by two independent observers.

Sample preparation

Serum samples were obtained by centrifugation of clotted blood and stored at −20°C until tested. Mononuclear cell preparations (MNC) containing lymphocytes and 5–15% monocytes were obtained by density centrifugation of heparinized venous blood. An aliquot of each preparation was depleted of monocytes by allowing their adhesion to a plastic culture dish. The resulting lymphocyte populations (PBL) contained less than 5% monocytes as assessed by esterase staining. Buffy coat populations containing lymphocytes, monocytes and polymorphonuclear leukocytes were obtained by dextran sedimentation of whole heparinized venous blood.

Auto-antibody screening

Sera were examined for the presence of antibodies to nuclear, smooth muscle, mitochondrial, gastric parietal and thyroid microsomal antigens by an indirect immunofluorescence procedure described elsewhere (Ockhuizen *et al.*, 1982). In brief, serum diluted 1:10 was incubated with 3-μm cryostat sections of rat liver, kidney and stomach and of human thyroid at 4°C for 20 min. After two washes in phosphate-buffered saline (PBS) pH 7·4, tissue sections were exposed to fluorescein isothiocyanate (FITC)-labelled $F(ab)_2$ fraction of goat antiserum to human IgG $F(ab)_2$ diluted 1:30 [fluorescein:protein (F:P) ratio 2 μg:1 mg, Cappell Laboratories, Cochranville, Pa.] at 4°C for 20 min, washed three times, mounted in 70% glycerol in PBS pH 7·8 and examined for fluorescence using a Zeiss Universal Microscope fitted for epi-illumination with a 75 W xenon light source and appropriate filter system. Where positive results were obtained, tests were repeated using doubling dilutions of sera to obtain the auto-antibody titre. Known negative sera were included in each run.

Auto-antibodies to thyroglobulin were detected by use of a haemagglutination method (Thymune-T, Wellcome Laboratories, Research Triangle Park, N.C.). Turkey erythrocytes coated with human thyroglobulin were incubated with sera diluted 1:10 to 1:5120 for 1 h at 22°C in microtitre wells and examined for haemagglutination. Controls included reaction of indicator cells with known positive sera and of uncoated erythrocytes with sera under test.

T lymphocyte enumerations

Total T lymphocytes were identified by the E rosette method (Goust *et al.*, 1980), in which PBL were centrifuged for 5 min at 250 ***g*** with a thirty-fold excess of sheep erythrocytes coated with aminoethylthiouronium bromide and allowed to react at 4°C for 10 min. The cells were gently resuspended and examined by microscopy for the percentage of lymphocytes forming rosettes with three or more erythrocytes.

Active T lymphocytes were counted by the method of Wybran & Fudenberg (1971). In brief, PBL were centrifuged for 5 min at 200 ***g*** with a ten-fold excess of uncoated sheep erythrocytes, immediately resuspended and examined for percentage of rosette-forming cells. This population of lymphocytes is responsible for reactivity to the mitogen concanavalin A and to allogeneic cells (Robbins *et al.*, 1981).

Interactive T lymphocytes were identified by their ability to form rosettes with Raji (human B lymphoblastoid) cells, as previously described (Goust *et al.*, 1980). In brief, Raji cells were centrifuged with a twenty-fold excess of PBL at 4°C for 5 min at 200 ***g*** and incubated for 1 h at 4°C, gently resuspended and examined for the percentage of Raji cells rosetting with three or more lymphocytes.

Helper-inducer and cytotoxic-suppressor T-lymphocyte subsets were identified by the use of the monoclonal mouse antisera Leu 3a and Leu 2a, respectively (Becton Dickinson Monoclonal Antibody Center, Sunnyvale, Calif.) (Ledbetter *et al.*, 1981) in a micro-immunofluorescence method described elsewhere (Galbraith *et al.*, 1980). In brief, PBL were serially incubated with mouse antiserum at 1:30 dilution and FITC-labelled Ig fraction of goat antiserum to mouse IgG at 1:50 dilution (F:P ratio 2 μg:1 mg, Cappell Laboratories) at 4°C for 5 min in microtitre wells, with intervening washes. After the final wash, cells were resuspended in 70% glycerol in PBS pH 7·8 and examined for fluorescence. The percentage of cells fluorescing with each antiserum was calculated and a helper:suppressor ratio derived.

Response to mitogens

The ability of lymphocytes to incorporate labelled precursor into DNA in response to stimulation with the mitogens phytohaemagglutinin (PHA) and concanavalin A (ConA) was examined as previously described (Galbraith *et al.*, 1980). In brief, MNC were cultured in triplicate with and without different doses of PHA and ConA in microtitre plates (5×10^6 cells per well) in RPMI 1640 medium with 10% fetal calf serum supplemented with L-glutamine, at 37°C for 72 h in a humidified atmosphere of 5% CO_2 in air. Twelve hours before termination of culture, the cells were pulsed with 1 μCi tritiated [^{3}H] thymidine and examined for ^{3}H incorporation by beta counting at 72 h. Results were expressed as mean raw c.p.m., mean net c.p.m. (c.p.m. of culture with mitogen − c.p.m. of culture in medium alone) and stimulation indices (mean c.p.m. of culture with mitogen ÷ mean c.p.m. of culture in medium alone).

Leukocyte migration inhibition factor (LIF) production by lymphocytes was investigated by the direct method of Spitler & Von Muller (1977). Briefly, triplicate aliquots of $1{\cdot}2 \times 10^7$ buffy coat cells were incubated with and without different doses of PHA in medium RPMI 1640 with 10% horse serum at 22°C for 90 min, then immediately placed in wells punched in agarose plates and incubated for 20 h at 37°C in a humidified atmosphere of 5% CO_2 in air. The area of cell migration from each well was calculated, and results expressed as follows:

$$\text{Migration index} = \frac{\text{mean area migration of cells exposed to PHA}}{\text{mean area migration of cells exposed to medium alone}}$$

Results were considered positive if a PHA dose response was observed and at least one migration index was $< 0{\cdot}8$.

Data analysis

Quantitative results, including cell percentages and ratios, c.p.m. ^{3}H incorporation, and stimulation indices were analysed for goodness of fit to a normal distribution. Patient and control populations were then compared by Student's *t* distribution test for two means. Auto-antibodies were considered to be either present or absent, and their frequency of detection in each population was compared by Fisher's exact *P* test. Similarly, LIF production was considered to be either positive or negative as described above and results compared by Fisher's exact *P* test.

RESULTS

Serum auto-antibodies

Auto-antibodies to thyroid antigens were detected in the sera of eleven of the thirty-two patients with alopecia areata (31%) and twelve of the twenty-eight with totalis/universalis (43%), and in one of the sixty control sera. Positive sera contained antibodies to either thyroid microsomal antigens alone (titre range: 40–640) or to human thyroglobulin alone (titre range: 80–1280), or to both, in almost equal distributions (Table 1). The majority (83%) of sera containing these antibodies were from female patients; however, Fisher's exact *P* analysis revealed no significant increase of frequency of such auto-antibodies in female as compared with male patients in this study.

TABLE 1. Serum auto-antibody distribution in patients with alopecia areata

	Anti-thyroid microsomal	Anti-thyroglobulin	Both antibodies	Total
Male (n = 18)	2 (11·1%)	1 (5·6%)	1 (5·6%)	4 (22·2%)
Female (n = 42)	5 (11·9%)	5 (11·9%)	9 (21·4%)	19 (45·2%)
Total (n = 60)	7 (11·6%)	6 (10%)	10 (16·7%)	23 (38·3%)

Anti-nuclear antibodies were detected in one male patient; all sera were negative for mitochondrial, smooth muscle and gastric parietal antibodies.

T-lymphocyte populations

The distributions of total, active, interactive, helper-inducer, suppressor-cytotoxic T-cell populations and helper : suppressor cell ratios were normal. Comparisons of mean percentages of cell populations and mean helper : suppressor ratios revealed no significant differences between total patient and normal control groups (Table 2). Similarly, no differences were found between male and female patients, or between either sex and the control group (data not shown). However, when patients were grouped according to disease severity and the presence or absence of circulating auto-antibodies to thyroid antigens, differences in the interactive T-cell populations (those rosetting with B lymphoblastoid cells) were found (Table 2).

Thus, patients with alopecia totalis or universalis were found to have significantly reduced percentages of interactive T cells (mean 20·6%, s.e. 1·4) when compared with the control population (mean 24·9%, s.e. 1·2, $P<0{\cdot}05$), as did patients with thyroid auto-antibodies (mean 20·5% s.e. 1·9, $P<0{\cdot}05$). This difference reached even greater statistical significance when patients with alopecia totalis/universalis and concurrent thyroid auto-immunity were compared

TABLE 2. Peripheral blood T lymphocyte populations in alopecia areata

	Total %	Active %	Interactive %	Helper (Leu 3^{+}) %	Suppressor (Leu 2^{+}) %	Helper/suppressor[2] ratio
Controls (n = 60)	70·7 (0·9)[1]	35·6 (1·7)	24·9 (1·2)	50·9 (2·5)	23·7 (1·3)	2·1 (0·1)
Patient groups						
Total (n = 60)	67·4 (1·0)	33·5 (1·4)	22·4 (1·4)	47·0 (2·2)	23·4 (1·6)	2·3 (0·2)
AA (n = 32)	67·3 (1·4)	34·2 (1·6)	24·2 (2·1)	48·8 (2·3)	23·9 (2·6)	2·4 (0·4)
ATU (n = 28)	67·5 (1·5)	32·8 (2·3)	20·6 (1·4)[3]	45·2 (3·7)	23·0 (1·9)	2.2 (0·3)
Thy. ab.$^{+}$ (n = 23)	68·3 (1·6)	35·9 (3·4)	20·5 (1·9)[3]	44·1 (2·9)	23·2 (2·2)	2·3 (0·4)
ATU/Thy. ab.$^{+}$ (n = 12)	67·5 (1·9)	37·5 (2·6)	18·7 (1·9)[3]	40·4 (4·6)	23·1 (2·5)	2·0 (0·4)

[1] All results expressed as mean value, with standard error of the mean (s.e.m.).

[2] Expressed as mean of individual ratios obtained.

[3] Values significantly reduced in comparison to controls.

Abbreviations: AA = patients with alopecia areata; ATU = patients with alopecia totalis or universalis; Thy. ab.$^{+}$ = patients with serum auto-antibodies to thyroid antigens; ATU/Thy. ab.$^{+}$ = patients with alopecia totalis/universalis and thyroid auto-antibodies.

with controls (mean 18·7%, s.e. 1·9, $P < 0\cdot01$). Analysis of data derived from these patient populations revealed no differences in total or active T-cell populations, or in helper : suppressor cell ratios (Table 2).

Response to mitogens

No significant differences in lymphocyte DNA synthesis in response to PHA or ConA were found between the total patient and control populations, or between patient groups delineated on the basis of disease severity or the presence of auto-immune phenomena when such groups were compared with controls or with each other (data not shown). These data were analysed in three ways as described above (raw c.p.m., net c.p.m., stimulation indices).

In contrast, LIF production in response to PHA stimulation was deficient (negative response) in a significant number of patients with alopecia totalis ($P = 0\cdot002$), of those with thyroid auto-reactivity ($P = 0\cdot007$), and of those with both ($P = 0\cdot003$), whereas there were no significant differences between the control group and the total patient population ($P = 0\cdot89$) or those with areata lesions ($P = 0\cdot15$). LIF data are shown in Table 3. LIF production was evaluated in forty

TABLE 3. PHA-dependent LIF production

	Positive	Negative
Controls (n = 40)	35	5
Patient groups		
Total (n = 40)	25	15
AA (n = 21)	16	5
ATU (n = 19)	9	10
Thy. ab.$^{+}$ (n = 14)	7	7
ATU/Thy. ab.$^{+}$ (n = 7)	2	5

Abbreviations as in Table 2.

of the sixty patients only. This was considered to be representative inasmuch as nineteen (48%) of these forty patients had alopecia totalis as opposed to twenty-eight (47%) of the total patient population.

DISCUSSION

Our immunological evaluation of sixty patients with alopecia areata revealed strong evidence that this disease is associated with aberrations in cell-mediated immunity when there is coexistent auto-immunity to thyroid antigens, when the alopecia involves total scalp or body hair, and especially when auto-immune phenomena and severe alopecia coincide. The changes in cell-mediated immunity were limited to reductions in 'interactive' T lymphocytes and deficient mitogen-dependent lymphokine production.

One of the major objectives of this study was to attempt to resolve the confusion arising from previous immunological studies of alopecia areata, many of which included only small numbers of patients. An example of this conflict is that whereas there have been reports of reduced numbers of peripheral blood T cells (Brown *et al.*, 1977; Gianetti *et al.*, 1978; Nunzi, Hamerlinck & Cormane, 1980) and deficient lymphocyte transformation responses to microbial antigens (Friedmann, 1981a), other studies have failed to reveal such defects in this disease (Gu *et al.*, 1981; Filipovitch *et al.*, 1982). In the present study, normal circulating total and active T cells and normal mitogen-dependent DNA synthesis (though decreased formation of mitogen-stimulated lymphokines) were found in the total population of sixty patients and also in the subgroups delineated by specific characteristics such as disease severity and auto-reactivity.

Another facet of alopecia areata which has provoked numerous investigations is the role of auto-reactivity in this disease. The association between alopecia areata and auto-immunity, particularly toward thyroid antigens, has been both reported (Cunliffe *et al.*, 1969; Friedmann, 1981a,b) and refuted (Main *et al.*, 1975; Klaber & Munro, 1978; Muller, Rook & Kubba, 1980). Conflicting data suggesting auto-reactivity to hair follicles has also been presented (Muller *et al.*, 1980; Nunzi *et al.*, 1980). Nevertheless, the current consensus appears to be that auto-immune phenomena are an important, though poorly understood feature of this disease.

In the current study we found a high prevalence of serum antibodies to thyroid antigens (alopecia areata 31% and totalis/universalis 43%) in the absence of clinical or chemical evidence of thyroid disease. This prevalence is similar to that recorded by Friedmann (1981b). However, perhaps greater importance lies in our finding of normal helper : suppressor T-cell ratios in these patients. Since the intriguing demonstration of suppressor T-cell deficiency in systemic lupus erythematosus (Sagawa & Abdou, 1978), many investigators have assumed that all auto-immune diseases must be characterized by a similar defect. A number of recent studies of alopecia areata have revealed increases (Gu *et al.*, 1981; Filipovitch *et al.*, 1982) and decreases (Ledesma & York, 1982) in suppressor T cells (in study populations of sixteen, fourteen, and nine patients, respectively). Although the results of these studies were statistically significant, the clinical relevance is less clear, particularly in view of our normal findings in a much larger patient population.

Our data show that aberrant immunity is not a characteristic feature of all patients with alopecia areata but the demonstration of deficiencies in interactive T cells and mitogen-dependent lymphokine production, which have not to our knowledge been previously investigated in this disease, suggests that cell-mediated immune mechanisms play a role in at least one form of alopecia areata. The interactive T lymphocyte (or 'B lymphoblastoid cell-rosetting T cell') population is comprised of a majority of cells bearing helper-inducer (Leu 3a$^+$, OKT4$^+$)

membrane markers and a small proportion of cytotoxic-suppressor cells (Leu $2a^+$, $OKT8^+$) (Goust & Fudenberg, 1983). Reductions in this population in peripheral blood are frequently associated with deficient lymphokine production and are characteristic of chronic viral, fungal, and mycobacterial infections and of certain diseases of suspected viral aetiology such as Behçet's syndrome (Fudenberg *et al.*, 1980; Galbraith & Fudenberg, 1983).

The relationship between the pathogenesis of alopecia areata and these defects in cell-mediated immunity is a matter for speculation, but three major possibilities may be considered. First, the immune defect may be related primarily to the thyroid auto-immune process. This appears unlikely for although significant reductions in interactive T cells and deficient lymphokine production were found in the totalis/universalis group of patients, 55% had no evidence of thyroid auto-reactivity.

Second, it is possible that the immune defects are secondary to the alopecia areata disease process: the apparent association of defects with severity of disease would support this. However, this association is also compatible with the third major possibility, that deficiencies in cell-mediated immunity may be involved in the cause of alopecia areata in certain patients. This last hypothesis is supported by results obtained from our preliminary trials of immunopotentiation therapy (Galbraith & Fudenberg, 1983) in which patients with alopecia totalis of several years duration responded not only with enhancement of cell-mediated immunity, but also regrowth of hair.

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Increased ratio of helper to suppressor T cells in alopecia areata

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SUMMARY

We studied lymphocyte subsets in the blood of twenty-four patients with alopecia areata of various degrees of severity. There was an increase in the ratio of helper to suppressor cells that correlated with the extent of the disease, providing further evidence that the immune system may be involved in its pathogenesis.

Several studies have suggested that alopecia areata may be the result of an immunological defect, though the possibility of it being a heterogeneous syndrome cannot be dismissed (Rook, 1977). The condition occurs more frequently in patients with other disorders that are thought to be autoimmune in origin e.g. Addison's disease (Irvine, Stewart & Scarth, 1967), thyroid disease (Cunliffe *et al.*, 1969), vitiligo, and lupus erythematosus (Muller & Winkelmann, 1963). It also seems to be associated with Down's syndrome in which T-cell dysfunction is frequent (du Vivier & Munro, 1975). Some workers have observed a high incidence of auto-antibodies to thyroid constituents, gastric parietal cells (Kern *et al.*, 1973; Friedmann, 1981a), and smooth muscle (Main *et al.*, 1975), though others have not confirmed this (Cunliffe *et al.*, 1969; Betterle *et al.*, 1975).

More direct evidence for an autoimmune aetiology in alopecia areata is the presence of a mononuclear cell infiltrate around the lower portions of hair follicles in the early stages of the disease (Muller, Rook & Kubba 1980), and the occurrence of antibodies to endothelium of the hair bulb capillary network upon circulating lymphocytes (Nunzi, Hammerlinck & Cormane, 1980). Leclesma & York (1982) found a reduced number of suppressor T cells in diffuse alopecia areata which might favour the induction of an immune response to a hair-associated antigen, but Hordinsky-Kramarczuk *et al.* (1981), using the same technique, found a high proportion of suppressor cells in three of eight patients with alopecia areata.

In order to resolve this discrepancy, we measured T-cell subsets in peripheral blood of patients with partial alopecia areata (AA) and with the more severe forms i.e. totalis (AAT) and universalis (AAU). Patients were screened for auto-antibodies to thyroid components, gastric parietal cells, smooth muscle and reticulin to see whether their occurrence was associated with an imbalance in T-cell subpopulations.

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METHODS

Twenty-four patients were studied. Seven had lost hair in patches (all female), ten had alopecia totalis (five female, five male), and seven had alopecia universalis (five female, two male). The mean ages of these three groups were 36·1 (range 17–58), 38·4 (14–64), and 43·3 (32–55) years respectively. A control group of seventeen females and four males with a mean age of 34·2 (15–63) was selected from healthy hospital staff. Three cases of partial alopecia were in the active stage of the disease (1–4 months of hair loss) and the rest had a more chronic condition of 1 to 35 years duration. None of the patients were receiving any form of medication apart from one with alopecia universalis who had been given 3 weekly injections of Synacthen®.

Mononuclear leukocytes were isolated from venous blood on Ficoll-Hypaque, and total T cells, helper cells and suppressor cells identified with OKT3, OKT4, OKT8 monoclonal antisera (Ortho diagnostic systems) followed by fluorescein-labelled goat anti-mouse serum (Meloy). The procedure was exactly as recommended by Ortho. For each test at least 300 cells were scored with a × 60 objective. When normal mouse serum was used as a control in place of monoclonal antibody, non-specific staining was usually below 3% and never exceeded 5%. Statistical analysis was by Student's *t*-test except for H/S ratios for which analysis of variance was used.

RESULTS

Lymphocyte and total T-cell counts were normal for all patients (Table 1). A decrease in suppressor T cells (OKT8$^+$) and a rise in helper T cells (OKT4$^+$) was progressively more noticeable with increasing severity of the disease. Suppressor cells were significantly depressed in both AAT ($P < 0·05$) and AAU ($P < 0·01$), but the change in helper cells reached statistical significance only in AAU ($P < 0·01$).

The ratio of helper to suppressor cells (H/S ratio) was calculated (Fig. 1). The H/S ratio increased progressively with the extent of hair loss, being significantly raised in AAT ($P < 0·01$) and AAU ($P < 0·001$).

A moderately high titre of auto-antibodies to thyroid components was found in four patients and a low titre in three others. This represents an incidence of 29%. A further two patients had

TABLE 1. Lymphocyte, T cell and T-cell subset counts in peripheral blood of patients with alopecia areata. Mean ± standard deviation

	Controls n = 21	AA patches n = 7	AA totalis n = 10	AA universalis n = 7
H/S ratio	1·79 ± 0·43	2·45 ± 0·93	2·85 ± 1·17**	3·36 ± 0·74***
Suppressor cells (%)	29·[illegible] ± 6·2	23·73 ± 6·[illegible]	22·53 ± 9·02*	17·26 ± 2·68**
Helper cells (%)	49·84 ± 5·98	54·0[illegible] ± 8·[illegible]2	55·[illegible]2 ± 7·63	57·13 ± 6·92**
Total T cells (× $10^3/\mu l$)	1·34 ± 0·40	1·38 ± 0·25	1·39 ± 0·42	1·11 ± 0·25
Lymphocytes (× $10^3/\mu l$)	1·88 ± 0·59	1·96 ± 0·46	2·08 ± 0·80	1·65 ± 0·38

*P 0·05.

**P 0·01.

***P 0·001

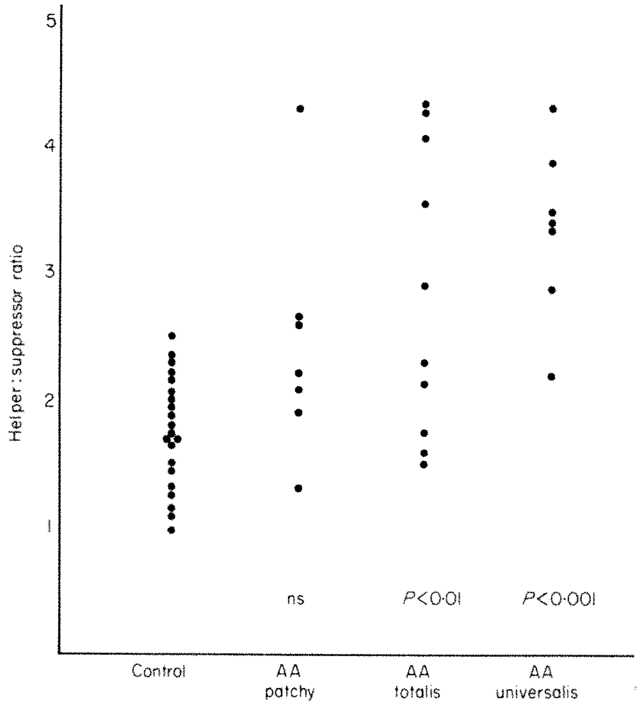

FIGURE 1. Ratio of helper (OKT4$^+$) to suppressor (OKT8$^+$) T cells in alopecia areata.

low anti-reticulin antibody levels and one showed weak anti-smooth muscle activity. A high H/S ratio was found in only two of the seven patients with thyroid antibodies, and in four out of ten with any type of auto-antibody.

DISCUSSION

In alopecia areata we have found a decreased number of suppressor cells together with a small increase in helper cells. The increase in the ratio of helper to suppressor cells correlated with the extent of hair loss. A reduced suppressor cell count has been seen previously by Leclesma & York (1982) in diffuse alopecia areata.

These results appear to support the concept of an autoimmune aetiology for AA, since a high H/S ratio is a common feature in autoimmune disorders, and it might facilitate an immune response to hair-associated antigens. Nevertheless, the evidence for an autoimmune mechanism in AA is not overwhelming. No investigator has been able to demonstrate lymphocyte reactivity *in vitro* to hair components, nor auto-antibodies in affected scalp by direct immunofluorescence (Kern *et al.*, 1973; Bystryn, Orentreich & Stengel, 1979; Igarashi, Takenshi & Sato, 1980; Friedmann, 1981b), although Nunzi and co-workers (1980) reported that small amounts of antibody to hair bulb capillary endothelium could be eluted from circulating lymphocytes. Furthermore, a lack of suppressor cell function is not consistent with lymphocyte hyporeactivity to mitogens in AA (Becker & Buckley, 1975; Herzer *et al.*, 1979), or with poor skin response to contact allergens (van Neste *et al.*, 1980).

Perhaps the imbalance between helper and suppressor cells in the blood does not represent a net deficit of suppressor function, but results from migration of suppressor cells into the skin.

The activity of helper T cells, which normally predominate in the epidermis (Patterson & Edelson, 1982), would then be counteracted, thereby inhibiting delayed type hypersensitivity. The way in which suppressor cells inhibit helper cells is not clear, but a cytotoxic action may well be involved (Pichler, Gendelman & Nelson, 1979), and it has been suggested that the cytotoxic potential of these cells is directed against hair follicle epithelium in alopecia areata (D'Ovidio, Vena & Angelini, 1981; Leclesma & York, 1982). An alternative to this cytotoxic hypothesis should be considered because there is no obvious damage of follicular structures in AA but the hair bulbs become arrested in early anagen phase (van Scott, 1958; Pierard & De La Brassine, 1975). Since it has now become apparent that helper T cells interact with epidermis (Patterson & Edelson, 1982) and can stimulate proliferation and functions of lymphocytes, macrophages, osteoclasts and fibroblasts (Horton *et al.*, 1972; Cohen, Pick & Oppenheim, 1979; Reinhertz *et al.*, 1980), it could be postulated that normal helper T-cell activity is required for the growth or maturation of the hair matrix.

Finally, there is the possibility that the H/S ratio is an early manifestation of an associated but undiagnosed autoimmune condition, e.g. thyroiditis. The incidence of auto-antibodies in the present study is comparable to that in a large survey by Friedmann (1981a) in which 24% of alopecia cases had thyroid antibodies compared with 7% for a control population. However, in our patients, a high H/S ratio did not correlate well with the presence of auto-antibodies.

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Collagen metabolism in two rare forms of epidermolysis bullosa

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SUMMARY

The collagenase activity in skin fibroblast cultures from three patients with rare forms of epidermolysis bullosa was assayed before and after proteolytic activation of the medium. Two of the patients had the recessive dystrophic form of the disease (REBD), which is generally associated with abnormal collagenase activity. The other patient had an atrophic mitis form of the disease (REBA), which has not previously been associated with defective collagen metabolism. However, a similar increase in collagenase activity was found in all three cases.

The total collagen production of EB fibroblasts was also enhanced, being two to five times that of control cell lines, and the intracellular hydroxylases of collagen biosynthesis were higher in the case of two EB-cell lines. These changes reflect the compensatory increase in collagen synthesis which follows the increased degradation caused by excessive free collagenase activity. Diphenylhydantoin treatment of one REBD patient for 9 months improved her condition.

Considerable progress in the understanding of the pathogenesis and mechanism of epidermolysis bullosa has been made during the last decade. This inherited disorder of the skin can now be divided into at least sixteen subtypes based on the clinical picture, the cleavage level of the skin and the pattern of inheritance (Gedde-Dahl, 1981). Recessive dystrophic epidermolysis bullosa (REBD) is one form in which the pathogenesis and the biochemical basis of the disease have been intensively studied. This form is characterized by trauma-induced blistering of the skin and mucous membranes resulting in heavy scarring, milium formation and in some cases severe deformities, especially in the distal extremities. The disease is seriously debilitating and often fatal. Ultrastructural studies originally indicated an association between the blistering and dermal collagen destruction (Briggaman & Wheeler, 1975; Kahl & Pearson, 1967) and subsequent investigations *in vivo* (Bauer, Gedde-Dahl & Eisen, 1977) and *in vitro* (Bauer & Eisen, 1978) suggested that REBD skin fibroblasts secrete increased amounts of collagenase, an enzyme that initiates collagen degradation (Eisen, Jeffrey & Gross, 1968). In the case of some patients with REBD, however, little or no increase in this enzyme could be found in tissue culture (Lazarus, 1972). These findings suggested the possibility of genetic heterogeneity in the

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disease. In one patient, the collagenase was shown to be structurally aberrant (Stricklin & Bauer, 1981) and in two an increased concentration, or a preferential translation, of collagenase mRNA was demonstrated (Kronberger *et al.*, 1982).

The anticonvulsant diphenylhydantoin has been shown to inhibit collagenase (Eisenberg, Stevens & Schofield, 1978), and this drug offers one potential medication for dystrophic forms of EB (Bauer & Cooper, 1981; Bauer *et al.*, 1980). According to Gedde-Dahl (1981) the reports and unpublished experimental work suggest that about half of the patients should respond to this drug.

We now report on collagenase activity and changes in collagen production and the activities of four enzymes of collagen biosynthesis in skin fibroblast cultures from two Finnish REBD patients and in one Finnish patient with a rare junctional, atrophic mitis form of EB (REBA mitis). The outcome of diphenylhydantoin treatment in one patient with REBD is also reported.

METHODS

Details of the three EB patients included in this study are given in Table 1. The diagnoses were based on the family history, clinical findings, routine histopathology and electron microscope examination of skin biopsies. In all three cases, the biopsies were taken from the border of fresh skin blisters on the forearm using local anaesthesia with 1% lignocaine. The biopsies for the control fibroblast cultures were taken during therapeutic surgery of age-matched individuals in the Clinics of Surgery and Paediatric Surgery, Oulu University Hospital, Finland. Fibroblast cultures were established routinely from the skin biopsies. The cells were grown in 65-cm^2 plastic culture dishes using 10 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 50 μg/ml of ascorbate, 290 μg/ml L-glutamine and a penicillin 100 U/ml, streptomycin 100 μg/ml mixture. For the collagenase assays, the cell layer of confluent culture was washed twice with phosphate-buffered saline and cultured for 8 h in a serum-free medium. Aliquot portions of this medium were used directly for the assay, without concentration.

Collagenase activity was assayed using [^{3}H]proline-labelled collagen substrate prepared in freshly isolated chicken fibroblasts (Dehm & Prockop, 1971). The assay was performed as described earlier (Ryhänen *et al.*, 1982), the cleavage products of the initial collagenase digestion being degraded further with a mixture of trypsin and α-chymotrypsin. The degradation products can be separated from the remaining uncleaved collagen substrate by rapid filtration in trichloroacetic acid. The trypsin activation of latent collagenase was always carried out using several concentrations, varying from 0·1 to 2·0 μg per 50 μl of medium sample (Bauer *et al.*, 1975).

The neutral proteolytic (non-collagenolytic) activity was assayed from the combined cells and media. The confluent fibroblasts grown for 8 h without serum were scraped into 2 ml of medium, concentrated Tris-HCl buffer with NaCl was added to give a final concentration of 0·1 M Tris-HCl (pH 7·4, 4°C) 0·4 M NaCl, the suspension was sonicated and caseinolytic activity was assayed with azocasein as a substrate by a modified method of Starkey & Barrett (1976). The incubation was carried out at 37°C for 60 min at pH 8·5. The incubation solutions contained 0·1 ml of 4% azocasein in 10 mM Na-phosphate buffer (pH 7·0), 0·1 ml of 0·2 M borate buffer (pH 8·5) and 0·1 ml of sample. The reaction was stopped by adding 0·4 ml of 10% trichloroacetic acid, the precipitate was centrifuged and the optical density of the supernatant was assayed at

TABLE 1. Clinical characteristics of the patients

Patient	1 REBD mutilans	2 REBD inversa	3 REBA mitis
Age, sex	16, male	13, female	20, female
Family history	Sporadic	Sporadic	Two sisters with REBD dead during first year of life
Onset	Birth	Birth	Birth
Distribution	Generalized	Belt-like zone on the lower body and axillae	Generalized
Digital fusion	Severely mutilating	Mild	No
Nail dystrophy	Yes	No	Yes
Hair	Thin	Thin	Scarring alopecia
Teeth	Destroyed	Destroyed	Destroyed
Flexural contractures	Severe	Mild	No
Healing pattern	Dystrophic scars	Atrophic scars	Atrophic scars
Strictures and involvement of gastro-intestinal mucosal areas	Strictures of mouth and oesophagus	Mild strictures of oesophagus and difficulties in swallowing	Hoarseness of voice
Physical development	Small size	Small size	Normal
Anaemia	Severe	Severe	Moderate
Histology	Dermal blistering	Dermal blistering	Junctional blistering with hypoplasia of hemidesmosomes

366 nm. One unit of enzyme activity was expressed as OD_{366} per hour and the results are expressed per mg of protein. Protein assays were performed using Bio-Rad Protein Assay according to instructions provided by the manufacturer (Bio Rad Laboratories, California, U.S.A.).

The activities of intracellular enzymes of collagen synthesis were assayed as described by Myllylä & Kivirikko (1982). The cell number was calculated in a Bürker-Türk haemocytometer and confirmed by fluorometric DNA assay (Brunk, Jones & James, 1979).

Total collagen production per cell was determined by incubating the confluent fibroblast cultures in DMEM supplemented as described under Methods, except that the 10% fetal calf serum was replaced with 2% dialysed fetal calf serum and [^{14}C]proline (1 μCi/ml) for 24 h. After the labelling period the medium was collected, proteinase inhibitors were added, proteins were precipitated by adding 390 mg/ml ammonium sulphate, and after overnight stirring at 4°C, the precipitates were collected by centrifugation at 13,000 *g* for 30 min. The precipitate was solubilized into 0·1 M Tris 0·4 M NaCl (pH 7·4), dialysed against this same buffer and 20% of the material collected from one culture dish was used at a time for [^{14}C]hydroxyproline assay after hydrolysis *in vacuo* at 160°C in 6 N HCl for 16 h (Juva & Prockop, 1966).

Diphenylhydantoin treatment was started with a dose of 100 mg twice daily. The serum concentration was maintained above 8 nmol/ml and the dose was adjusted according to serum levels.

RESULTS

Collagenase activity

The Finnish patients with recessive dystrophic epidermolysis bullosa originate from a unique, isolated genetic pool. We measured the collagenase activity in their cultured skin fibroblasts to see whether the changes in activity were similar to those reported from other populations (Bauer & Eisen, 1978). One patient with the rare recessive atrophic mitis form of the disease (REBA) was also included in this study to see whether the changes in collagen metabolism differed from those found in REBD.

There was no significant difference in total (active plus latent) collagenase yield per cell in the cell lines of any patient compared with the controls. When medium collagenase was assayed directly, without activation, significantly higher activities could be found in the skin fibroblast cultures of both REBD patients (Fig. 1). In the second patient, as much as 80·6% of total collagenase was in the active form, and although the amount was lower in the first patient (38·5% of total activity), even this figure is still higher than the amount of free collagenase in the controls (5·4 ± 0·7%). Surprisingly, the fibroblasts of patient 3, with REBA, showed a very similar change in collagenase activity, the free collagenase activity being 62·3% (Fig. 1).

The neutral proteinase activity (caseinolysis) was not different in fibroblasts of any EB patient (patients 1–3: 0·31 U/mg ± 0·068) when compared with control fibroblasts (six lines: 0·32 U/mg ± 0·110).

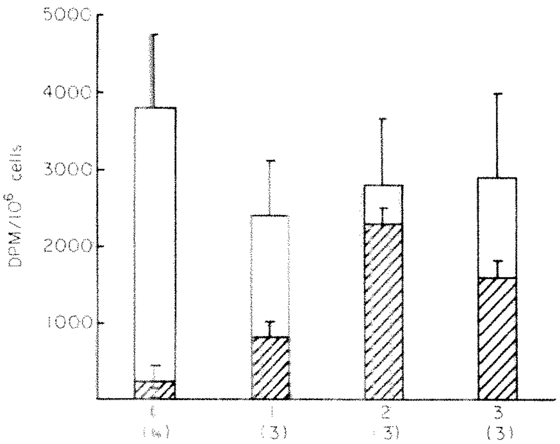

FIGURE 1. Collagenase activity expressed as DPM in culture medium of control (C) and three EB-cell lines (1–3). The values are expressed per 10^6 cells, the bars indicate the standard deviations, the hatched columns the activity directly assayed from the medium and white columns the maximal activity after proteolytic activation (see Methods). The numbers in parentheses express the number of cell lines in the case of controls and the number of assays in the case of patients.

TABLE 2. Rate of collagen production in cultured skin fibroblasts (DPM/10^6 cells)

Cell line	[^{14}C]hydroxyproline	[^{14}C]proline	[^{14}C]hyp / [^{14}C]pro
Control (n = 6) Mean ± s.d.	6468 ± 1946	53,309 ± 13,927	0·12 ± 0·01
REBD (1)	21,885	88,000	0·25
REBD (2)	14,869	60,700	0·24
REBA (3)	31,690	118,000	0·20

Collagen biosynthesis

Total procollagen production, expressed as total [^{14}C]hydroxyproline secreted into the culture medium in 24 h, is given in Table 2. All the EB lines showed a significantly higher production than the age-matched control cell lines. There was also a significant (over two s.d.s) difference in the total amount of [^{14}C]proline-labelled proteins, in the case of REBA fibroblasts and in one line of REBD fibroblasts, suggesting differences also in the secretion rate of proteins other than collagen. However, the ratio of hydroxyproline to proline, reflecting the ratio of collagen to non-collagenous proteins, was clearly increased in all three EB-cell lines.

Increased collagen production per cell was also indicated by significantly higher activities of prolyl-4-hydroxylase in the case of the one REBD (1) and one REBA (3) patient, whilst another hydroxylating enzyme of collagen biosynthesis, lysyl hydroxylase, also showed activities higher than those in the control cells. In contrast, the glycosyl- and galactosyltransferases, the two sugar transferases of collagen biosynthesis, were not significantly higher than in the controls (Table 3). The fibroblasts of the other REBD patient (2) did not show any increase in the enzyme activities, this same line also clearly showing the smallest increase in total collagen production (Table 2).

TABLE 3. The activities of four intracellular enzymes of collagen biosynthesis

Cell line	Enzyme activity (DPM/μg DNA)			
	Prolyl 4-hydroxylase	Lysyl hydroxylase	Glycosyl* transferase	Galactosyl* transferase
Control (n = 6) Mean ± s.d.	4·37 ± 1·20	18·36 ± 2·20	3·67 ± 0·94	4·33 ± 1·27
REBD (1)	10·92	40·7	5·47	6·28
REBD (2)	3·42	19·0	5·16	6·05
REBA (3)	8·00	29·5	3·52	4·33

* These enzymes are galactosyl-hydroxylysyl-glucosyltransferase and hydroxylysyl-galactosyltransferase, which glucosylate the hydroxylysine residues during post-translational modification of collagen.

Effect of diphenylhydantoin treatment

Only one of the two Finnish REBD patients was willing to try diphenylhydantoin as a medication for epidermolysis. No dramatic healing of the patient's skin was obvious upon inspection after 9 months' treatment, but the skin at the edges of the lesions had become thicker and many miliae had developed. The pharyngeal symptoms had also eased, bowel evacuation was easier, and according to the patient, her loss of hair had decreased. These promising observations have encouraged us to continue the treatment.

DISCUSSION

Earlier reports have recorded an increased synthesis of immunoreactive skin collagenase in several patients with REBD (Bauer & Eisen, 1978; Valle & Bauer, 1980), a phenomenon thought to be genetically unique to this form of EB (Valle & Bauer, 1980). Further analysis has shown increased synthesis of structurally abnormal collagenase (Stricklin, Welgus & Bauer, 1982) and enhanced cell-free translation of this enzyme in REBD patients (Kronberger *et al.*, 1982). We could not demonstrate any increase in total enzymatic collagenase activity in two Finnish REBD patients but found a constant increase in free collagenase, the enzyme form which can be directly measured from the culture medium. This finding is in agreement with previous work, in which an identical assay was used to study one REBD patient from the U.S.A. (Ryhänen *et al.*, 1982). It was not possible to determine whether the collagenase assayed in this study was enzymatically abnormal, because of the limited amount of enzyme available to us from cell cultures of a single skin biopsy per patient.

The increased amount of active collagenase found in both Finnish REBD patients, who originated from an isolated genetic pool, supports the idea that a collagenase defect is typical of REBD. A similar increase of free enzyme was also found in the only Finnish EB patient who had, clinically and electronmicroscopically, a very rare, atrophic mitis form of the disease. To our knowledge there are no earlier *in vitro* studies on collagenase in this form of EB. The distinctly abnormal ratios of active to total collagenase in the two REBD patients, taken together with this finding, suggest that in spite of genetic heterogeneity within the one form of EB, a common biochemical abnormality may exist between clinically different forms of this inherited skin disease.

The increased collagen production and the increased levels of prolyl and lysyl hydroxylases presumably reflect compensatory enhancement of collagen biosynthesis as a response to increased degradation by collagenase, since increased activity of the hydroxylases correlates well with increased collagen synthesis (Kivirikko & Myllylä, 1982).

Our findings thus suggest that the biochemical defect in the recessive dystrophic form of EB with a dermal cleavage level, and possibly also in some atrophic forms in which the cleavage level is at the dermo-epidermal junction, consists of increased release of free collagenase by the skin fibroblasts. This increased degradation seems to be coupled with enhanced synthesis of collagen. Both phenomena persist in cultured fibroblasts throughout several passages.

Since we were unable to demonstrate any increase in the amount of non-collagenolytic, non-specific proteolytic activity in any of the tested fibroblast cultures, it seems that the natural activators of the latent form of collagenase would not be increased, at least not to the extent which would explain the increase of free collagenase in EB cultures. The assay method used here (neutral caseinolysis) is not the most sensitive one and possibly does not detect all proteolytic activities. However, the complexity of the regulation of collagenase activity offers other explanations for the presence of excess active collagenase in EB cultures. These include at least two possibilities: a decrease in the amount of several inhibitors regulating the collagenolytic activity in extracellular matrix or a disturbed binding of these inhibitors to a possibly defective enzyme protein.

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Inhibition of dihydrotestosterone-mediated hamster sebaceous gland hypertrophy by progesterone

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SUMMARY

In the hairless hamster, progesterone can antagonize dihydrotestosterone-mediated hypertrophy of the sebaceous gland.

Although potentially an androgen precursor (Voigt & Hsia, 1973) progesterone acts primarily as an anti-androgen on androgen-responsive organs by competing for intracellular 5α-reductase and thereby inhibiting the conversion of testosterone (T) to dihydrotestosterone (DHT). This conversion step is undoubtedly of major importance for most organs which are under androgen control. However, the sebaceous gland may be unusual in that direct testosterone-mediated gland function, even in the presence of 5α-reductase blockade, seems possible. It has also been suggested that, even if such control does not occur, the effects of progesterone cannot be explained in terms of 5α-reductase inhibition alone. Evidence has appeared that progesterone, or its 5α-reduced metabolite, may compete with DHT for the androgen cytosol receptor (Wright, Kirchhoffer & Giacomini, 1980), but there seems to be no evidence that progesterone can antagonize the effects of DHT on sebaceous glands *in vivo*. The recent interesting communication of Vermorken, Goos & Wirtz (1982) underlines this point, since they found that topical progesterone did not prevent DHT-induced hypertrophy of the hamster flank organ. A similar result was reported by Girard & Barbier (1980) in terms of sebum excretion by the hamster flank organ.

In this context therefore, we would like to report that we have demonstrated consistently that progesterone, following i.c. injection, will antagonize DHT-mediated sebaceous gland hypertrophy in the hairless hamster.

The method is essentially as described elsewhere (Picton & Clark, 1977). Sebaceous glands on the dorsum of the female hairless hamster are used in preference to the flank organ. The animals, with body weights in the range of 100 ± 10 g, were used in groups of six. Two sites were marked

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TABLE 1. Sebaceous lobe volumes 4 days following i.c. injection. Values are quoted relative to the normal (unstimulated) gland volume, mean ± s.e.m.

Treatment	Relative sebaceous lobe volume
Control	1·00
1 mg progesterone i.c.	0·79 ± 0·10
1 mg DHT i.c.	3·04 ± 0·33
1 mg progesterone + 1 mg DHT i.c.	2·33 ± 0·16

Rank order; DHT > progesterone with DHT > progesterone = control (Kruskal-Wallis, Mann-Whitney tests; $P < 0·05$).

on the dorsum of each animal. Four groups were employed, being treated with either 0·1 ml of suspension fluid alone, 1 mg progesterone alone, 1 mg DHT alone, or 1 mg DHT + 1 mg progesterone, all drugs being administered in 0·1 ml suspension fluid. Sebaceous lobe volumes were measured 4 days after treatment. Results of a typical experiment are shown in Table 1, from which it is evident that, in this model at least, direct antagonism of the DHT response is possible with progesterone.

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The establishment and clinical value of a dermatological photobiology service in a district general hospital

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SUMMARY

The availability of a local dermatological photobiology facility has proved to be of great value in the management of patients with photodermatoses, especially those with chronic actinic dermatitis. It is our belief that clinically useful results can only be achieved if careful attention is paid to technical factors such as radiation measurement.

During the past two decades, interest in clinical dermatological photobiology has increased considerably. This interest has been due in part to the development of equipment, notably the irradiation monochromator, which is suitable for the investigation of photodermatoses. In the United Kingdom two centres have made important contributions to our present knowledge of photosensitivity disease, viz., the Department of Photobiology, Institute of Dermatology, London, and the Department of Dermatology, Ninewells Hospital, Dundee. The results of the photobiological investigations carried out at these two centres and factors relating to the classification, causation, and treatment of the various photodermatoses have previously been discussed (Magnus, 1976; Frain-Bell, 1979), as have technical factors associated with the design and performance of the irradiation monochromators that are used (Magnus *et al.*, 1959; Mackenzie & Frain-Bell, 1973).

The success achieved by these two centres has encouraged workers in other centres to invest in various types of photo-irradiation apparatus and set up their own investigative studies, and in this communication we report our own experience in initiating a dermatological photobiology service and discuss how the results of these investigations have influenced patient management. It is our belief based on this relatively short experience, that photobiological studies using equipment such as the irradiation monochromator will yield clinically useful results only if careful attention is paid to technical factors, particularly to radiation measurement.

Some of the clinical uses of the monochromator irradiation tests are (Magnus, 1974):

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(1) increased knowledge of whether the patient is photosensitive or not;
(2) determination of the specific spectral sensitivity (action spectrum);
(3) possible identification of the chromophore;
(4) clearer delineation of photodermatosis syndromes;
(5) characterization of 'new' diseases.

The acquisition of knowledge in *all* these five areas requires resources of manpower, expertise and time which are beyond those of most dermatology departments. In many instances, however, it may suffice to have information concerning points 1 and 2 for satisfactory clinical management of the patient. It has been our main objective to acquire data concerning these two points.

In this communication we provide information on the equipment and technical aspects of our photo-irradiation techniques, together with a brief summary of those photodermatoses which are most usefully investigated.

Administration of the photodermatology service

The photobiology laboratory is housed in the Regional Medical Physics Department, Dryburn Hospital, Durham. The protocol of photobiological testing for each patient is determined after discussion between the referring physician and the physicist, and the subsequent photo-irradiation is carried out by a photobiology technician. The service is used by Consultant Dermatologists and their junior medical staff from eleven Health Authorities who work in an area of approximately 30 miles radius around Durham City. Patients who attend the Medical Physics Department for photodermatological investigation return on the following day so that one of us (B.L.D.) can assess the delayed (24-h) minimal erythema doses (MEDs) and any morphological changes. A brief report of these delayed reactions, together with any reactions observed during or shortly after irradiation, is then sent to the dermatologist who requested the investigation. Occasionally patients who live within easy reach of the hospital and who are suspected of polymorphic light eruption (PLE) may attend on several days for repeated irradiation (Epstein, 1980). Currently about 150 patients with suspected photosensitivity are investigated per year.

Technical factors

The apparatus used for phototesting is the photo-irradiation system (model 497/02) manufactured by Applied Photophysics Limited, London.

The optical components in an irradiation monochromator deteriorate with time such that, in general, the irradiance at the patient's skin becomes less with increasing usage of the apparatus. Also, a decision regarding the degree of photosensitivity of an individual following phototesting involves a comparison with the published radiant exposures (or 'dose') of other workers achieving similar biological end-points (e.g., the MED). For these reasons routine measurement of irradiance (W m^{-2}) and prescription of radiation exposure in radiometric units (J m^{-2}) are essential (Magnus, 1976).

A full description of the photo-irradiation system, radiation dosimetry, and quality control is given in the appendix.

Irradiation protocol

Our objectives in investigating subjects with suspected photosensitivity are twofold: (i) to support, or otherwise, an initial diagnosis of photosensitivity, and (ii) to establish a crude action spectrum.

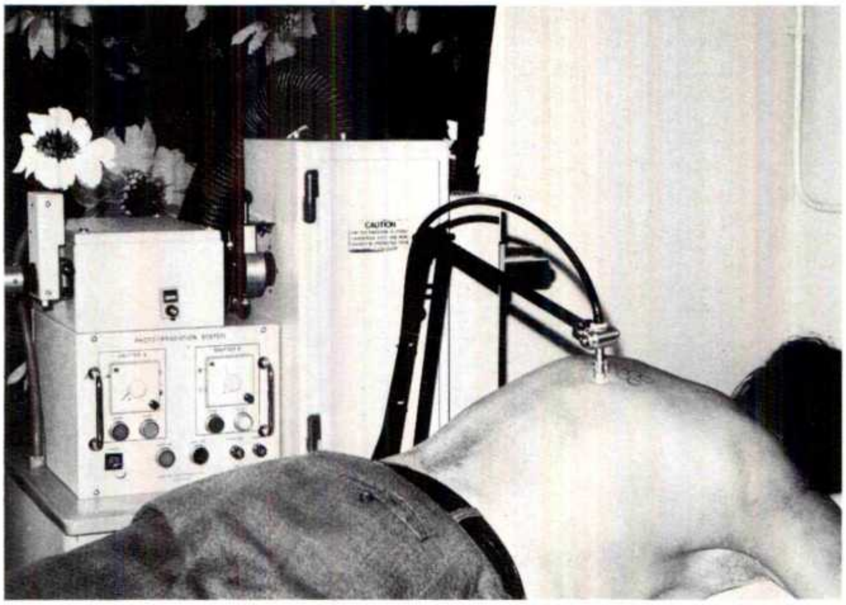

FIGURE 1. A patient being investigated for suspected photosensitivity with the irradiation monochromator. Note the liquid-filled light guide which is used to conduct the radiation from the exit slit of the monochromator to the patient's skin.

The patient normally lies prone on a couch during the investigation with a light guide, which conducts radiation from the monochromator to the patient's skin, resting lightly on the patient's back and supported on a flexible arm (an old 'Anglepoise' lamp) which slides along a rail attached to the side of the couch (Fig. 1). Our experience with the light guide has shown the results of phototesting to be more consistent than when the patients used to sit on a stool with their back against the exit port of the monochromator, since the patient is better able to relax lying down and the operator can easily see if the light beam moves off the desired irradiation site due to patient movement.

The spectral ranges that are adopted for routine phototesting are given in Table 1, although occasionally irradiation at other wavelengths is carried out if suggested by the patient's history. Dose increments based on a ratio of $\sqrt{2}$ are used in each spectral region; this geometrical series is equivalent to doubling alternate doses. A colour glass filter (Schott type WG335) is inserted into the proximal end of the light guide for irradiation in the UV-A and visible regions to eliminate any stray radiation with wavelengths less than 320 nm.

The normal ranges given in Table 1 for delayed (24-h) minimal erythema doses are compiled partly from our own unpublished observations using the technique of probit analysis (Mackenzie, 1983), and partly from recent published data (Mackenzie, 1983; Parrish, Jaenicke & Anderson, 1982).

In addition to monochromatic irradiation, patients suspected of PLE are often exposed to about three MEDs of solar simulating radiation (see appendix for details) on one or more days. The purpose of this irradiation is to observe whether abnormal morphological changes are produced, and to this end the irradiation site is inspected daily for several days following exposure (Jansen, 1982).

TABLE 1. A summary of the spectral regions normally used and the delayed (24-h) minimal erythema doses (MEDs) for normal subjects

Central wavelength (nm)	Full bandwidth at half maximum intensity (nm)	Median MED in normal subjects (approximate 95% confidence limits in parentheses) (J cm^{-2})
300	5	0·028 (0·014–0·056)
320	10	2 (0·7–5)
350	30	27 (14–64)
400	30	> 150

Clinical results

The results presented in Table 2 pertain to those patients under the care of a single Consultant Dermatologist (F.A.I.) who have been referred for routine phototesting during the past 2 years. As mentioned previously, the service is used by several other dermatologists, and in addition, special studies on drug-induced photosensitivity are carried out in conjunction with clinicians in other specialities.

A discussion of the results obtained in the various photodermatoses is given below. Of the fifty-two patients finally diagnosed as photosensitive, the laboratory phototesting indicated a positive result in 37 (71%) of them.

1. Chronic actinic dermatitis. Following the proposal of Ramsay (1981) we have chosen to adopt the term 'chronic actinic dermatitis' to include the three chronic photodermatoses of persistent light reactivity, actinic reticuloid and photosensitive eczema. Clinical opinion still varies, however, on the nosology of these three diseases.

TABLE 2. Numbers and diagnoses of patients referred for phototesting

Final clinical diagnosis	No. of cases	No. of positive phototest results
Idiopathic photodermatoses		
Chronic actinic dermatitis	22	20
Polymorphic light eruption	8	7
Solar urticaria	2	2
Actinic prurigo	4	1
Hydroa vacciniforme	1	0
Light aggravated dermatoses		
Atopic eczema	8	3
Psoriasis	2	0
Lupus erythematosus	1	1
Photocontact dermatitis and drug-induced photosensitivity	4	3
Others (non-photosensitive)	12	
Total number of patients tested	64	

Twenty (60%) out of a total of thirty-four patients referred for phototesting with a suggested clinical diagnosis of chronic actinic dermatitis demonstrated increased sensitivity to UV radiation or visible light.

On the basis of extreme photosensitivity to UV-B, UV-A and often visible light, together with clinical and, on occasions, histological appearance, a diagnosis of actinic reticuloid was made in seven patients.

Comprehensive photopatch testing was not performed. In a small number of cases, however, suspected photocontact sensitivity to musk ambrette has been investigated using a technique based on protocols described elsewhere (Raugi, Storrs & Larsen, 1979; Giovinazzo *et al.*, 1980). Our inability to search routinely for an exogenous photosensitizer has made it impossible to identify those patients who may be persistent light reactors. Despite this limitation, the ability to define an approximate action spectrum and to quantify the degree of photosensitivity has been clinically useful in that appropriate photoprotective agents can be chosen and sensible methods of UV radiation avoidance suggested. In those cases of severe chronic actinic dermatitis, in which patients often have to make radical alterations to their lifestyle, we regard phototesting to be essential for diagnosis and useful for the patient as it provides confirmatory and easily understood evidence of their photosensitivity.

It is interesting to note that fourteen patients who were initially diagnosed as possible chronic actinic dermatitis showed normal responses to phototesting. Upon further examination an alternative diagnosis, often an unsuspected contact dermatitis, was eventually found in all but two cases.

2. Polymorphic light eruption. Relatively few patients with this diagnosis have been phototested. This probably reflects awareness amongst referring physicians that normal or equivocal results are often obtained. In fact, seven out of eight patients referred with a clinical diagnosis of PLE demonstrated abnormal responses to phototesting. Low MEDs to UV radiation were found in four patients: two patients exhibited low MEDs to both UV-B and UV-A; one patient exhibited a low MED to UV-B only; one patient exhibited a low MED to UV-A only. A delayed papular response following exposure to solar simulating radiation was seen in four cases (three of whom had normal MEDs).

3. Other photodermatoses. As shown in Table 2 a number of patients with other idiopathic photodermatoses and dermatoses aggravated by light were also phototested. In many of these patients laboratory phototesting failed to confirm the clinical diagnosis. This is perhaps not surprising: attempts to reproduce hydroa vacciniforme experimentally have been disappointing (Magnus, 1976; Harber & Bickers, 1981); monochromatic irradiation tests give no abnormal results in about half of the patients with actinic prurigo (Calnan & Meara, 1977); in five girls with atopic eczema, phototesting with an irradiation monochromator produced no confirmatory evidence of abnormal responses to UV radiation or visible light (Frain-Bell & Scatchard, 1971).

CONCLUSION

In our experience the availability of a dermatological photobiology service has proved to be of great clinical value in the management of patients, especially those with severe chronic actinic dermatitis. Phototesting, however, is a time consuming procedure (both for the patient and technical staff) and in order that the service is not overloaded careful consideration should be given to the expected clinical value of the test in each individual case. Close liaison between the referring physician and medical physicist is essential for the appropriate choice of test and its correct interpretation.

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APPENDIX

Description of the photoirradiation apparatus

The apparatus used to irradiate the patients consists of a 900 W xenon arc lamp mounted on a housing with two exit ports at 180° to each other. One exit port is optically coupled to a single diffraction grating monochromator. A liquid-filled light guide, 2 m in length and 5 mm inner diameter, conducts the radiation from the exit slit of the monochromator to the patient's skin (Fig. 1). This light guide transmits radiation in the spectral interval 270 to 740 nm with 50% or more transmission in the region 300 to 600 nm.

The other exit port on the lamp housing is optically coupled by two fused silica lenses to a filter holder. This permits very high intensity radiation in broad spectral regions to be achieved. By inserting a single colour glass filter (Schott type WG305, 3-mm thick) into the light path, it is

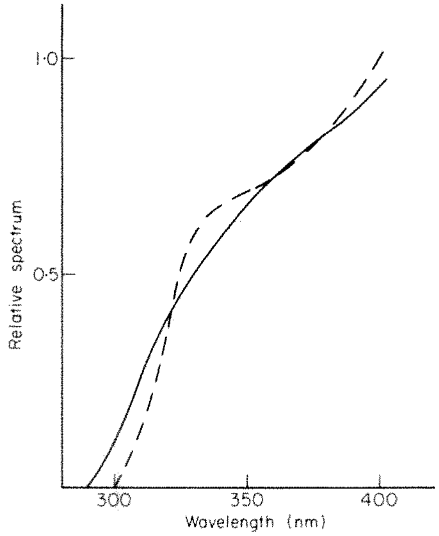

FIGURE 2. The relative ultraviolet spectrum of 'solar simulating radiation' (——) (xenon lamp with WG305 filter) and midday summer sunshine in the U.K. (- - - -). Both curves are normalized to give the same total UV power.

possible to produce a continuous spectrum of radiation from about 290 to 1200 nm. The spectrum of this radiation in the ultraviolet region has been determined spectroradiometrically using apparatus described elsewhere (Diffey & McKinlay, 1983) and is compared in Fig. 2 with the spectrum of sunlight at sea level for midday summer sunshine in the U.K. The patient is irradiated with about three MEDs of this solar simulating radiation in a circular beam of 25 mm diameter. The exposure time is estimated from the skin type of the patient and the actinic

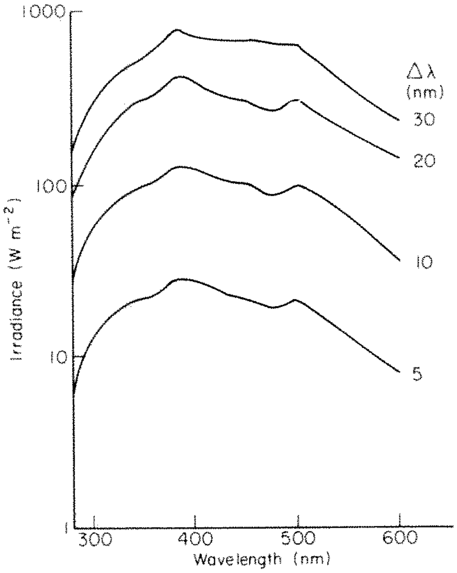

FIGURE 3. The variation of irradiance with wavelength at the patient end of the liquid-filled light guide for four different bandwidths (Δλ nm) after $2\frac{1}{2}$ years usage.

irradiance determined using a special UV detector which is sensitive only to wavelengths less than about 320 nm (IL730A actinic radiometer; International Light Inc., Newburyport, U.S.A.). The exposure time for three MEDs ranges from about 12 to 45 seconds as the skin type increases from I to IV.

Radiation dosimetry and quality control

The instrument which is used routinely to measure irradiance is a thermopile (model 2M; Dexter Research Center, Michigan, U.S.A.). This instrument has been found to be linear in response at irradiances up to 1000 W m^{-2}. It is compared every 6 months with our 'standard' thermopile (model FT17; Rank Hilger, Margate) which has been calibrated by the National Physical Laboratory, and so we estimate the uncertainty of our output measurements to be within ± 5% of the true irradiance. The measured irradiance at the end of the light guide in the plane of the patient's skin is shown in Fig. 3 for four different bandwidths over the spectral range 280 to 700 nm. These figures are those obtained after $2\frac{1}{2}$ years' use of the apparatus. In order that scientifically meaningful and clinically useful results are obtained after phototesting a patient, it is important to be confident of the characteristics of the radiation beam incident upon the patient. To this end, regular quality control checks are carried out which include measurement of the irradiance at the plane of the patient's skin over the required range of wavelengths and bandwidths, checking the wavelength calibration of the monochromator, checking the bandwidth and spatial uniformity of the exit beam, and estimating the magnitude of stray radiation. Details of these measurements can be found elsewhere (Diffey, 1982; Magnus, 1976; Mackenzie & Frain-Bell, 1973).

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The dermatology out-patient service: a study of out-patient referrals in a Scottish population

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SUMMARY

A comprehensive survey of the dermatology out-patient service, including private practice, was undertaken by the medical staff of the Department of Dermatology, in Edinburgh. The unit serves a population of 1,171,000 in the south-east region of Scotland.

One thousand two hundred and thirty-two new patients and 1587 follow-up patients were seen in 1 month. Ninety-five per cent of new referrals were from general practitioners and the remaining 5% were from hospital doctors. Benign tumours (19·3%), viral warts (16·9%) and dermatitis/eczema (16·7%) accounted for over half of all new consultations. The routine waiting time for a clinic appointment was 5 weeks, but 9·5% of new patients were seen within 3 weeks of referral and a further 6·5% within 48 hours. Minor surgical procedures, excluding the treatment of viral warts, were carried out on 22% of new patients.

A recent questionnaire sent to all members of the British Association of Dermatologists revealed that only half had taken part in more than three investigative studies during the previous 5 years. Many had wished to do so but their clinical commitments had not allowed enough time for research. Surprisingly, little has been written recently about the out-patient workload of British dermatologists. It seemed therefore that a study of the out-patient work of a group of dermatologists was overdue, particularly in view of the recommendations made in 1981 by the Dermatological sub-committee of the Central Committee for Hospital Medical Services (CCHMS) about the job description for consultant dermatologists, and of the guidelines issued by the Joint Committee on Higher Medical Training, regarding specialist training (JCHMT, 1982).

DERMATOLOGY SERVICES IN SOUTH-EAST SCOTLAND

The area served by the Department of Dermatology in Edinburgh includes Lothian Region, the Borders and a large part of Fife (Fig. 1). Ninety per cent of the population of 1,171,000 live

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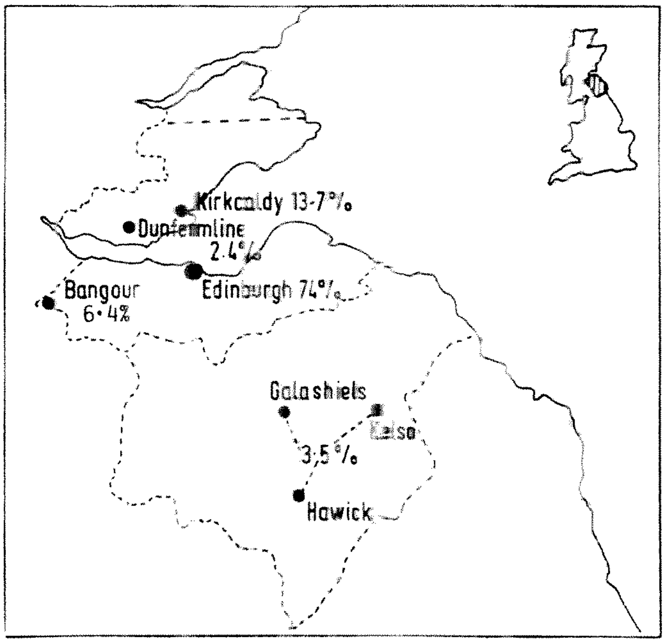

FIGURE 1. Area served by the Department of Dermatology in the south-east region of Scotland. Percentages represent proportion of the total of new patients seen during the survey.

within urban areas. The major unit is in the Royal Infirmary, Edinburgh, where four out of five of the consultants are based, the fifth working mainly in Fife. Clinics are held each week in other Edinburgh hospitals, as well as at District General Hospitals in West Lothian, Fife and the Borders (Fig. 1). In-patient facilities are available only in the Royal Infirmary, Edinburgh where there are forty-one beds in two dermatology wards. Formal dressing services are limited to two hospitals with the most clinics, the Royal Infirmary, Edinburgh and the Victoria Hospital, Kirkcaldy.

Every referral letter to our department is vetted daily by a doctor so that early appointments can be arranged when necessary. The more urgent appointments, from general practitioners or other hospital doctors, are arranged by telephone.

The medical complement of our department (corresponding NHS half-day sessions shown in parentheses) comprised one professor (5), four consultants (39), one associate specialist (6), two senior registrars (15), two registrars (20), two clinical assistants (6) and one house physician (10) whose main responsibility is to in-patients. Three private consultation sessions in Edinburgh are provided by two of our consultants. There is no other private dermatological practice in the region.

All staff participate in undergraduate and postgraduate teaching, and most in some form of research. Groups of 7–9 medical undergraduates attend the department in the Royal Infirmary in 2-week rotations for 46 weeks of each year.

METHODS

During 1 month (November, 1981) an intensive effort was made by all members of staff to keep accurate records, and the following were recorded

(a) The total number of new and follow-up patients seen in out-patient clinics, in other hospital departments and in private practice.

(b) The demand for early or urgent appointments at a time when our routine waiting time was 5 weeks.

(c) The diagnosis for each patient.

(d) Treatment and investigative procedures.

(e) The distribution of workload between established staff and trainees.

RESULTS

One thousand, two hundred and thirty-two new patients and 1587 review patients were seen (new:review = 1:1·3). Diagnoses were recorded in all but 2·8 of the new patients and 0·9% of review cases. The most common diagnostic groups for new and return patients are shown in Table 1.

TABLE 1. Distribution of diagnoses of new and review patients, for a 1-month period

Diagnoses	New patients (1232)	Review patients (1587)
Benign tumours	19·3%	6·1%
Viral warts	16·9%	33·0%
Dermatitis/eczema	16·7%	19·0%
Infections	7·1%	2·6%
Psoriasis	6·7%	10·0%
Malignant tumours	5·3%	4·1%
Acne/rosacea	4·4%	4·2%
Miscellaneous	23·6%	21·0%

Surgical procedures, investigations and dressings

Biopsies were performed in 13·4% of new patients and 1·4% of return patients (total of 188). Minor procedures including curettage, cautery, triamcinolone infiltrations etc. were carried out in 8·8% of new patients and 6·5% of review patients (total of 213).

In addition, cryotherapy to viral warts was peformed in 16·9% of new patients and 33% of review cases. Many of these patients were seen in two special wart clinics held each week in the Royal Infirmary, Edinburgh.

Non-surgical investigations e.g. blood tests, bacteriological, mycological and radiological tests were performed in 10% of all cases. Four per cent of new patients had dressings carried out by nursing staff in the out-patient departments.

Early/urgent appointments

Over 9% of new cases were considered, either by ourselves or by their general practitioners, to merit early appointments, i.e. within 3 weeks or less, compared with the routine waiting time of 5 weeks. Urgent appointments were arranged for a further 6·5% of new cases, who were seen on the same day or within 48 hours. Thirty-seven per cent of these requests came from other hospital departments.

In the urgent group, one-third of patients had dermatitis, and a further one-third had infections, urticaria, allergic conditions or vasculitis. In the group seen at early appointments, tumours, both benign and malignant, dermatitis and psoriasis accounted for 60% of the diagnoses.

Distribution of clinical workload

Consultants conducted an average of four general clinics each week and most had in addition a special clinic, for example pigmented lesions, contact dermatitis and atopic eczema. Staff in training posts conducted an average of three general clinics per week plus one minor surgery session and a share of the special clinics or wart clinics.

TABLE 2. Average number of N.H.S. patients seen per month, excluding wart clinic patients

	New	Review	Rate of review (of new patients)
Consultant	131	10[illegible]	30%
Senior registrar	95	4[illegible]	29%
Registrar	58	12[illegible]	64%

Table 2 records the average number of patients, excluding wart clinic patients, seen per month by each group. Registrars arranged review appointments for new patients twice as often as consultants or senior registrars.

DISCUSSION

The assessment of one's own clinical work can be painful and ego-threatening, but there is no better way to judge the efficacy of clinical activity. Human nature makes most of us believe that we are doing more than we really are: if it achieved nothing else, our study destroyed that illusion! It also made us *think* about our out-patient service—evolved during the last 100 years, and inherited rather than developed.

In the National Health Service decisions are apt to be made by anonymous committees divorced from the reality of humdrum practice; and these committees depend too often on facts culled from poor recording systems, so that their planning becomes irrational. Deficiencies were certainly found in our own records system. For example, the number of new patients was underestimated by 10%, mostly because ward consultations were not recorded in the official returns. On the other hand the number of review patients was overestimated by 60%, as our records system did not separate out those patients attending for daily dressings, and not necessarily being seen by a doctor. This distinction becomes important when estimates of the out-patient nursing establishment have to be made, and of course the value of an efficient out-patient dressing service is immense (Vickers, 1977). At the time of writing, the cost to the Health Service of a single out-patient visit to the Royal Infirmary, Edinburgh is £4·30, in contrast to the £106 needed to keep a patient in the ward for 1 day.

The range of diagnoses of the new out-patients in this study differs little from other reports from the United Kingdom between 1968 and 1976 (Rook & Wilkinson, 1979a), apart from an excess of benign tumours, which topped our list at 19·3%. This must reflect the high prevalence of these tumours in the community (Rea, Newhouse & Halil, 1976). Interestingly, Rook & Wilkinson (1979b), have commented on the increasing number of patients with benign naevi referred to out-patient clinics, often because of anxiety about possible malignant change. Apart from benign naevi, seborrhoeic warts and simple papillomata were the most common lesions in our survey. This may be a sign of the greater desire of our patients for what is essentially cosmetic surgery. It also reflects our willingness to treat them, which in turn overburdens our minor surgical sessions.

One must be careful when comparing the prevalence of conditions in a population with hospital referral rates. The latter are affected by many variables including the facilities offered by local general practitioners, the nature of the population (urban or rural), the social class distribution and the availability of specialist clinics. Nevertheless useful information can be obtained. For instance, the diagnoses of our new patient referrals differ from those recorded in the Lambeth (London) survey (Rea *et al.*, 1976) on the prevalence of skin disorders in two main respects. Viral warts accounted for 16·9% of our new referrals but for only 3·4% of the skin disorders noted in the population of Lambeth. However, the Lambeth survey was restricted to adults. Our special wart clinics in the Royal Infirmary seem not to discourage general practitioners in Edinburgh from treating warts, as the proportion of our new patients with warts is no greater than those of other dermatology departments (Rook & Wilkinson, 1979a). Moreover, provision of liquid nitrogen for some general practitioners has even led to a decline in wart referrals to some of our clinics.

Patients with acne vulgaris accounted for only 3·7% of the new referrals to our clinic compared with 8·6% of all skin diseases seen in Lambeth, evidence perhaps that general practitioners find the modern treatment of acne effective and easy to administer. The relatively few patients with acne vulgaris referred to our clinic contrasts sharply with the 20·1% of new patients with acne who are seen in office practices in the U.S.A. (Prevalence, Morbidity and Cost of Dermatological Diseases, 1979). This highlights the main difference between office practice in the U.S.A., where patients refer themselves to a dermatologist (primary referral) and practice in the U.K. where patients are seen initially by their general (family) practitioner or another hospital doctor, before secondary referral to a dermatologist.

We have noticed a steady increase in the number of minor surgical procedures performed in our out-patient clinics, and the finding that one in five of the new patients had such a procedure carried out during their first visit was not a surprise. We estimate that at present one unit of medical time is required to carry out minor procedures and to remove blood for investigations for every two units devoted to general dermatological consultations. Most of the surgical and investigative procedures are at present carried out by our junior staff. Staff in training, while requiring experience in surgical and other treatment procedures, should also see a reasonable number of new and review patients preferably under the supervision of a consultant (JCHMT, 1982). If, however, as recommended in the Short Report (Social Services Committee, 1981), consultant numbers increase at the expense of junior staff, then consultants may have to accept a greater share of the surgical workload. The recent recommendations of the Central Committee for Hospital Medical Services (CCHMS) propose that one consultant should serve a population of between 150,000 and 200,000.

Six and a half per cent of new cases were seen as a result of an urgent request from a general practitioner or another hospital department. Most opinions were given within 24 hours and all within 48 hours. One in three of these requests came from hospital departments. As hospital medicine becomes more specialized, fewer doctors in other wards seem prepared to diagnose and treat skin conditions without the advice of a dermatologist. Although the number of patients in this category was not large, a disproportionate amount of time was spent on such ward consultations which take place in hospitals throughout the region. They should not be forgotten in a dermatologist's job description.

In Edinburgh general practitioners have always been encouraged to telephone a consultant or duty registrar if an urgent consultation is required, and such patients are usually seen at the clinic on the same day. On the other hand, domiciliary visits have never been popular and only 0·5% of new referrals were seen at home during our study month. This may be one of the lowest rates in the country.

In 1957 our medical staff was less than one half of its present number. The current establishment has to be justified by looking closely at the changing practice of dermatology. The overall number of out-patients seen at the Royal Infirmary has altered little in the past 25 years. In 1957 new out-patients ran at 660 per month compared with the 680 of our sample month. However, more clinics are now held in peripheral hospitals, where, on average, another 420 new appointments are made each month. As some of these hospitals are many miles from Edinburgh (range 12–53 miles), staff travelling time is considerable.

We now conduct special clinics for patients with unusual or severe conditions: a good example is the treatment of recalcitrant psoriasis, with photochemotherapy, cytotoxic agents or retinoids. These patients take up a lot of time as their therapeutic regimes need careful monitoring. There is no doubt that we spend more time now looking after patients with severe skin conditions for which there was no effective treatment 30 years ago. In addition, patients now have more knowledge of their condition, and as a result ask for details and time-consuming explanations of their problems. Finally patients, doctors, nurses and other ancillary staff may not now be prepared to participate in huge, unending clinics. But despite all these facts there remains a nagging suspicion that Parkinson's first law might also be operating: 'Work expands to fill the time available for its completion'.

Many recommendations have been made to improve the standard of training in our specialty. The Whitley Council regulations entitle consultant staff to 30 days study leave every 3 years, senior registrars to 1 day each week and 30 days over 3 years, and registrars to 1 day each week during term time or 30 days each year. To make this feasible, without weakening the clinical service, an increase in staff is required. We calculate that we need nearly one whole time equivalent (WTE) of medical staff just to cover the statutory study leave requirements of our staff (10 WTEs)!

We have between seven and nine undergraduate medical students attached to our department in the Royal Infirmary for 46 weeks in each year, and their education is a heavy burden on a relatively small unit. In addition no teaching department worth its salt is without visitors and elective undergraduates from abroad. Post-graduate attachments and a steady flow of general practitioners through the department must also be anticipated. Similarly our staff give regular lectures to nurses, chiropodists, dental students and so on, and are also invited to give talks throughout the country and occasionally abroad. As a result the university/teaching department must be staffed at a higher level than a unit in a non-teaching hospital. This factor is usually underestimated when considering job descriptions.

We receive frequent requests to open new clinics in other hospitals. The obvious advantage, that patients do not have to travel long distances, may often be outweighed by disadvantages less apparent to other doctors and administrators. The nature of the out-patient clinic is too often misunderstood, but clearly a good clinic depends on numerous special facilities including minor surgery, daily dressings and more recent developments such as photochemotherapy. In our view, in a city of the size of Edinburgh (population 450,000) it is preferable for the patient, and also economic good sense, to concentrate resources on one well-appointed and well-staffed department, where undergraduate and post-graduate students can also gain good clinical experience.

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Expression of differentiation antigens in mammary and extramammary Paget's disease

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SUMMARY

Seven cases of mammary and twelve cases of extramammary Paget's disease were studied for the presence of carcinoembryonic antigen (CEA) and apocrine epithelial antigen (AEA) using the immunoperoxidase technique. CEA was found in the Paget cells in five out of seven mammary and in all twelve cases of extramammary Paget's disease, whereas the AEA reaction was positive in six mammary and all extramammary cases. The same antigens were also found in the cells of intraductal or ductal adenocarcinomas of the breast associated with mammary Paget's disease, suggesting a common origin for the cells. In the cases of extramammary Paget's disease studied no underlying malignant neoplasms could be detected. Our findings support the suggestion that Paget cells originate in sweat gland ducts and undergo an apocrine differentiation.

Carcinoembryonic antigen (CEA) was originally found in fetal tissues and in certain gastro-intestinal neoplasms (Gold & Freedman, 1965). Later it was demonstrated in epidermoid carcinomas (Lindgren, Wahlström & Seppälä, 1979) and adenocarcinomas of the uterine cervix (Wahlström *et al.*, 1979), in epithelial cells of eccrine and apocrine sweat glands and ducts (Penneys, Nadji & McKinney, 1981) and in cells of benign and malignant sweat gland tumours (Penneys, Nadji & Morales, 1982a; Penneys *et al.*, 1982b).

Human milk contains fat globules surrounded by the apical membrane of the lactating mammary epithelium. Antisera raised against the milk fat globule proteins have recently been shown to react with normal and malignant mammary epithelium (Ceriani *et al.*, 1977; Imam, Laurence & Neville, 1981) and also with sebaceous and apocrine sweat gland epithelium (Heyderman, Steele & Ormerod, 1979) with rather high specificity.

The atypical cells (Paget cells) found in the epidermis in mammary and extramammary Paget's disease are thought to originate from the epithelium of mammary and sweat ducts respectively (Jones, Austin & Ackerman, 1979). In order to clarify the origin and differentiation

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of Paget cells we have studied seven cases of mammary and twelve cases of extramammary Paget's disease by immunohistochemical staining with antisera against CEA and milk fat globule glycoprotein.

METHODS

Seven cases of mammary and twelve cases of extramammary Paget's disease were studied. After radical mastectomy an intraductal or invasive ductal adenocarcinoma of the breast was found in every patient with mammary Paget's disease. Three of the patients also had metastases in axillary lymph nodes. The site of the lesions of extramammary Paget's disease are shown in Table 2. No underlying carcinomas of the sweat glands or deeper structures could be detected clinically in any of these patients.

The skin biopsies or surgically removed preparations of both mammary and extramammary Paget's lesions were fixed in buffered 4% formaldehyde, processed routinely and embedded in paraffin. In six cases of mammary Paget's disease specimens from intraductal or ductal adenocarcinoma were also studied.

Antisera

Rabbit anti-CEA serum was obtained from Dako Immunoglobulins (Copenhagen, Denmark) and was thoroughly absorbed with a perchloric acid extract of human spleen as described previously to ensure a specific reaction with CEA only (Lindgren *et al.*, 1979).

Rabbit antiserum against milk fat globule glycoprotein, which we call apocrine epithelial antigen (AEA), was prepared in the following way. Human milk fat globules were isolated by centrifugation and the membrances separated from the butter oil by repeated extractions with phosphate-buffered saline (PBS) at 50°C. The membrane preparation was solubilized in 1% Triton X-100 in PBS and the glycoproteins were isolated by passage over a concanavalin A–sepharose column. The retained glycoproteins were eluted by alpha-methyl-pyrano-mannopyranoside. To obtain a more distinct reaction with the formalin-fixed preparations, the isolated glycoproteins were treated with 4% formaldehyde in PBS and dialysed overnight against PBS. Rabbits were repeatedly immunized with 400–500 mg of formalin-treated glycoproteins emulsified in Freund's adjuvant and the resulting antisera were absorbed before use with erythrocytes and normal blood leukocytes from buffy coats of 400 ml blood. The detailed characterization of the anti-AEA antiserum will be reported elsewhere (Forsman *et al.*, in preparation).

Immunoperoxidase staining

Histological sections, 4-μm thick, were deparaffinized in xylene and washed in PBS. Endogenous peroxidase activity was destroyed by incubating the sections with methanol containing 0·3% of hydrogen peroxide at room temperature for 30 min, after which the sections were washed in PBS containing 2% normal rabbit serum. The test sections were incubated in moist chambers with 1:100 diluted anti-CEA or anti-AEA sera at 37°C for 60 min. After washing with PBS the sections were incubated with biotinylated anti-rabbit IgG (Vectastain®, ABC kit, Vector Laboratories, Inc., Burlingame, CA, U.S.A.) at 37°C for 30 min, washed with PBS and incubated with avidin and horseradish peroxidase (Vectastain®) at 37°C for 30 min. After washing again the colour reaction was developed by incubating the sections with a solution containing 0·04% 3-amino-9-ethyl-carbazole and 0·01% hydrogen peroxide in 0·05 mM sodium acetate buffer, pH 5·0, at 37°C for 15 min. After washing in running tap water the coverslips were mounted with Aquamount® (Gurr, Hopkin & Williams Ltd., Chadwell Heath, U.K.).

Control sections were treated in a similar manner, but the anti-CEA serum was fully absorbed with purified CEA (Lindgren *et al.*, 1979) or it was replaced by either normal rabbit serum or PBS. The anti-AEA serum was similarly absorbed with AEA for control stainings. In other controls, each of the following reagents were replaced with PBS. No counterstain was added to the immunoperoxidase-treated sections, but adjacent sections were stained with haematoxylin and eosin.

RESULTS

The clinical data and the results of the immunoperoxidase staining for CEA and AEA in mammary Paget's disease are shown in Table 1. The intraepidermal Paget cells were positive for CEA and AEA in five and six cases, respectively. The CEA-positive material was located in the cytoplasm of the cells, while AEA was mostly seen at the surface membrane (Fig. 1). The intensity of the reaction varied in different cases. In one case of mammary Paget's disease the Paget cells were totally negative for both antigens. In CEA- and AEA-positive cases the cells in intraductal or ductal adenocarcinoma of the breast and in lymph node metastases were also positive for CEA and AEA, respectively (Fig. 2). The normal epithelium of mammary ducts contained AEA (Fig. 2c), but not CEA (Fig. 2b).

The results in extramammary Paget's disease are shown in Table 2. In all twelve cases the Paget cells were distinctly positive for both CEA (Fig. 3) and AEA, even if the intensity of the staining for AEA was weaker. The adjacent keratinocytes were always negative for both antigens. CEA could be demonstrated also in the epithelium of normal eccrine and apocrine sweat glands and ducts, and AEA in the epithelial cells of sebaceous and apocrine sweat glands.

TABLE 1. Clinical features and results of immunostaining for CEA and AEA in mammary Paget's disease

Patient	Sex	Age	Site of Paget cells	Immunostaining for CEA	Immunostaining for AEA
1	Female	60	Areola	–	–
			Breast cancer	nd	nd
2	Female	59	Areola	+	+
			Breast cancer	+	+
3	Female	55	Areola	+	+
			Breast cancer	+	+
4	Female	57	Areola	–	+
			Breast cancer	–	+
5	Female	53	Areola	+	+
			Breast cancer	+	+
6	Female	79	Areola	+	+
			Breast cancer	+	+
7	Female	58	Areola	+	+
			Breast cancer	+	+

nd = not determined.

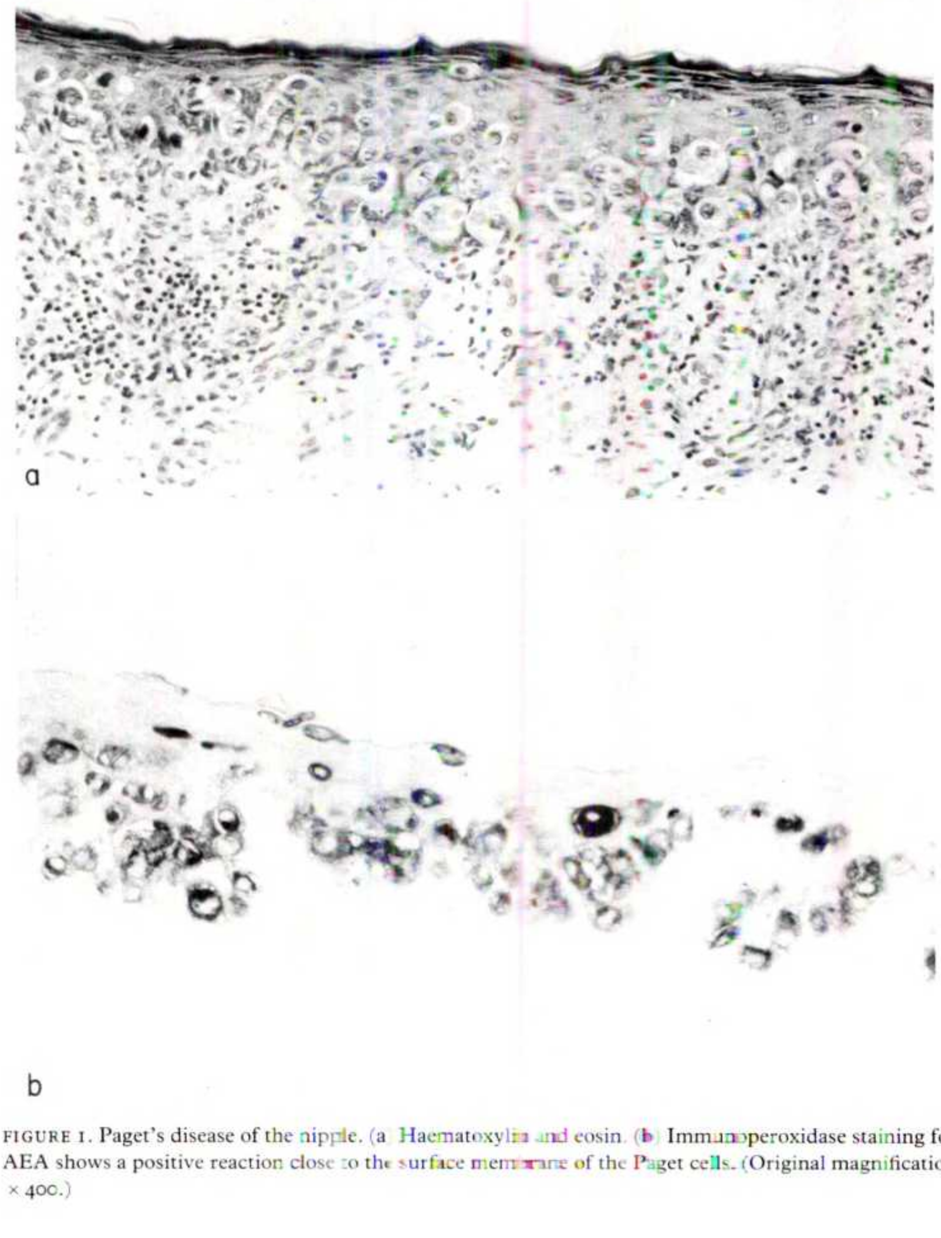

FIGURE 1. Paget's disease of the nipple. (a) Haematoxylin and eosin. (b) Immunoperoxidase staining for AEA shows a positive reaction close to the surface membrane of the Paget cells. (Original magnification × 400.)

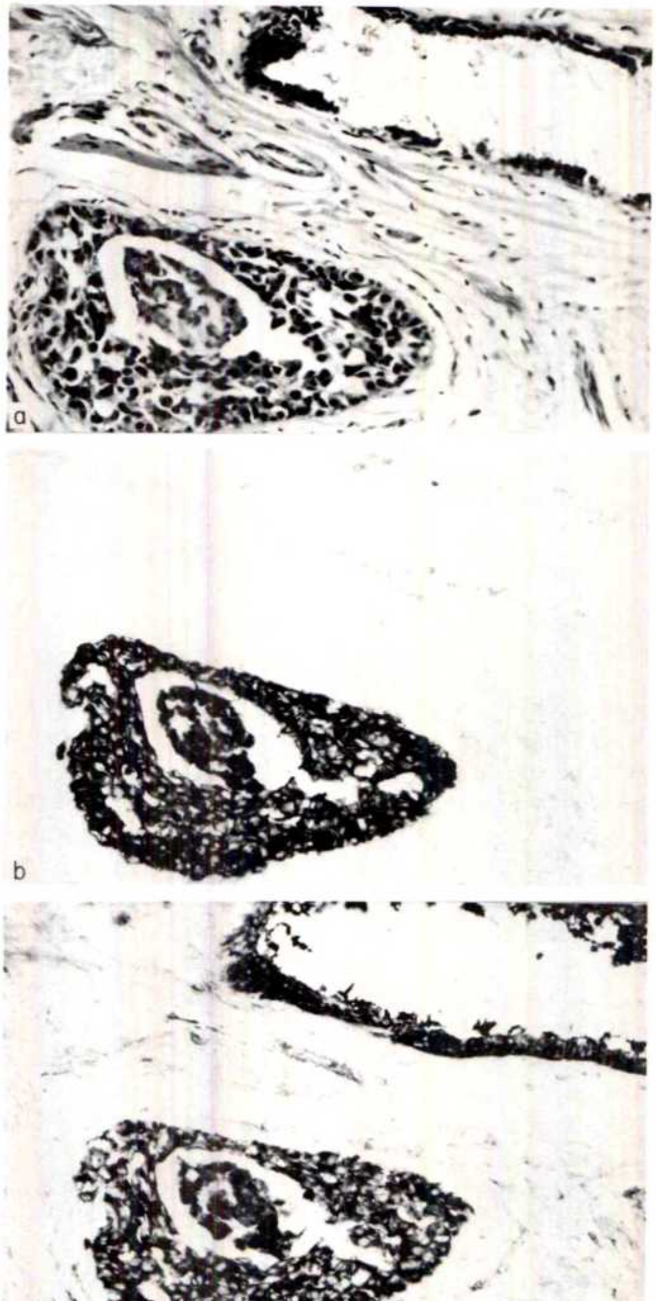

FIGURE 2. Intraductal adenocarcinoma of the breast. (a) Haematoxylin and eosin. (b) Immunoperoxidase staining for CEA shows a positive reaction in adenocarcinoma cells, but not in the normal ductal epithelium. (c) The staining for AEA is positive in both adenocarcinoma cells and normal ductal epithelium. (Original magnification × 400.)

TABLE 2. Clinical features and results of immunostaining for CEA and AEA in extramammary Paget's disease

Patient	Sex	Age	Location of the Paget's lesion	Immuno-staining for CEA	Immuno-staining for AEA
1	Female	34	Vulva	−	+
2	Female	72	Vulva	−	+
3	Female	63	Vulva	−	+
4	Female	63	Vulva	−	+
5	Female	79	Vulva	−	+
6	Female	70	Vulva	−	+
7	Female	68	Groin	−	+
8	Male	70	Groin	+	+
9	Male	89	Groin	+	+
10	Female	56	Pubes	+	+
11	Female	73	Pubes	+	+
12	Female	54	Axilla	+	+

DISCUSSION

The origin of the atypical cells found in the epidermis in mammary and extramammary Paget's disease is obscure. Paget's disease of the breast is almost always associated with an intraductal or ductal adenocarcinoma of the breast, and an underlying adenocarcinoma of sweat gland, gastrointestinal or genitourinary origin is also found in some cases of extramammary Paget's disease (Jones *et al.*, 1979). Most cases of extramammary Paget's disease however have been confined to the epidermis with no detectable malignancies (Jones *et al.*, 1979).

Various theories about the histogenesis of Paget cells have been presented. In carcinoma-associated cases they are thought to represent an epidermal manifestation of the underlying neoplasm, although it is not known whether they are transformed keratinocytes or infiltrating adenocarcinoma cells derived from the primary tumour (Nadji *et al.*, 1982; Jones *et al.*, 1979). Recently, Nadji *et al.* (1982) have shown CEA in Paget cells in all of seven cases of mammary Paget's disease. In our series two cases were CEA-negative. Using the immunoperoxidase technique CEA has been demonstrated in human breast carcinomas, but the incidence of detection has varied in different studies (Heyderman & Neville, 1977; Walker, 1980). Further, no correlation between the occurrence of CEA and the biological behaviour of the cancer has been found (Walker, 1980). The finding that both the intraepidermal Paget cells and adenocarcinoma cells of the primary tumour were equally CEA-positive or negative suggests a common origin.

Primary and metastatic carcinomas of the breast almost always express AEA (Ceriani *et al.*, 1977; Sloane & Ormerod, 1981), but in our experience, detection of this antigen seems to be related to the differentiation of the carcinoma cells. Occasional anaplastic carcinomas are AEA-negative while virtually all of the common ductal carcinomas stain strongly with the anti-AEA serum (Forsman *et al.*, unpublished). In our series the Paget cells were AEA-negative in only one case of mammary Paget's disease. Unfortunately, specimens from the primary tumour were not available for study.

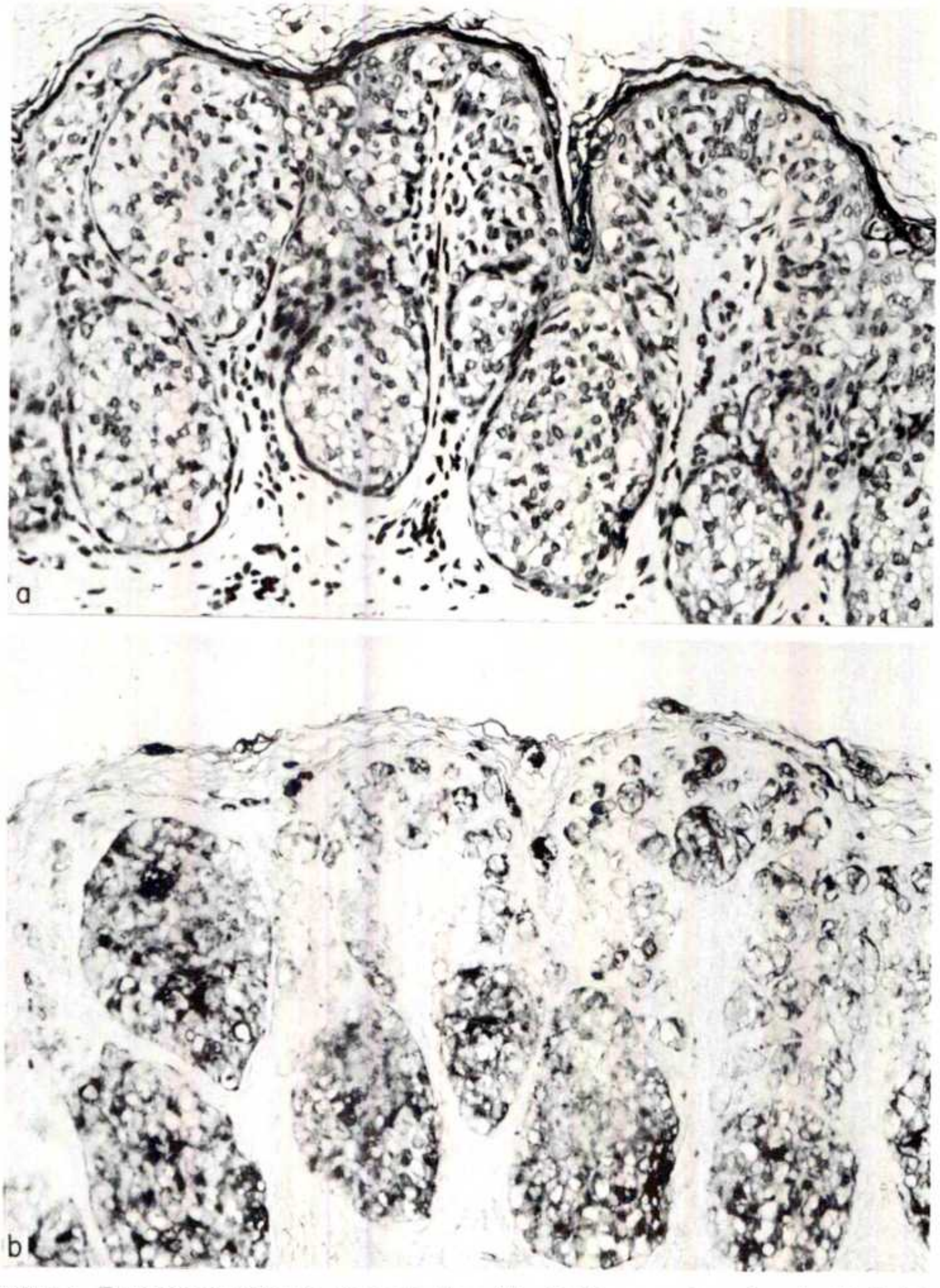

FIGURE 3. Extramammary Paget's disease in the axilla. (a) Haematoxylin and eosin. (b) Positive immunoperoxidase staining for CEA. (Original magnification × 400.)

In line with the results of Nadji *et al.* (1982), the Paget cells in all cases of extramammary Paget's disease in our study were positive for CEA and also for AEA. No underlying carcinomas were found in the patients. Electron microscopic studies suggest that the Paget cells in these cases are secretory epithelial cells (Belcher, 1972) possibly derived from pluripotential germinative epidermal cells (Jones *et al.*, 1979) or from the poral epithelium of eccrine or apocrine sweat ducts (Nadji *et al.*, 1982). The presence of both CEA and AEA in Paget cells speaks for the sweat duct origin and apocrine differentiation of these cells in extramammary Paget's disease.

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Brief Communication

Serum carcinoembryonic antigen level in Paget's disease

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SUMMARY

Serum carcinoembryonic antigen (CEA) levels were examined in ten patients with genital Paget's disease, using radioimmunoassay. Three of the patients showed elevated serum CEA levels, but the other seven patients had normal levels. All three patients with increased CEA levels had widespread metastases. The seven patients with normal levels had no metastasis or had only a very small metastasis in one lymph node. Elevated serum CEA levels are associated with Paget's disease only when it shows widespread metastases.

Carcinoembryonic antigen (CEA), originally described as a biomarker for colonic and gastric adenocarcinoma (Gold & Freedman, 1965), has subsequently been found in association with many other neoplasms (Denk *et al.*, 1972; Marchand *et al.*, 1975; Wahren, Edsmyr & Zimmerman, 1975; Velde *et al.*, 1982). CEA has great potential clinical value as a systemic marker of tumour activity. As a monitor of tumour burden, CEA can show early evidence of tumour recurrence or response to treatment.

Nadji *et al.* (1982) have found histochemically the presence of CEA in both mammary and extramammary Paget cells. In a previous paper (Furue *et al.*, 1983), we have confirmed the presence of CEA in Paget cells of the skin, and in the metastatic lesions of extramammary Paget's disease. We also found one patient with elevated serum CEA levels who had widespread metastases.

The present study was designed to further assess the relationship between elevated serum CEA levels and Paget's disease.

METHODS

We studied ten patients with genital Paget's disease. The diagnosis was based on histological examination. At the time of the first serum sampling, none of these patients had received immunotherapy or chemotherapy. In some patients the serum CEA level was checked subsequently. There were six males and four females, aged 50 to 78 years. An estimation of tumour burden was checked extensively including lymphangiography. Inguinal lymph node resection was performed in all patients and these lymph nodes were checked histologically for the presence of metastases.

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After collection all sera were stored at −20°C until use. Serum CEA levels were determined by radioimmunoassay, using the sandwich technique as previously described (Furue *et al.*, 1983). Briefly, the serum of a patient and a disk with previously attached anti-CEA antibody were incubated, so that CEA in the serum combined with the disk. ^{125}I-labelled anti-CEA antibody was incubated with the disk. The level of bound ^{125}I-labelled anti-CEA antibody within the disk was then determined using a gamma scintillation counter. The amount of CEA present in the sample was determined from a standard curve. The normal CEA level ranged from 0 to 2·5 ng/ml.

RESULTS

Three of ten patients showed elevated serum CEA levels, but seven patients had normal values of CEA ($<2{\cdot}5$ ng/ml) (Table 1). All three patients who showed elevated serum CEA levels had widespread metastases. In case 10, who showed a markedly high CEA level, widespread bone and lung metastases were noted roentgenographically although autopsy was not done. Two patients (cases 8 and 10) showed higher CEA levels as their metastatic lesions became more widespread (Table 1). Six patients with normal CEA levels had no sign of metastasis, but one patient (case 9) with a normal CEA level had a very small metastasis in one lymph node.

TABLE 1. Serum CEA levels in genital Paget's disease

Case	Age	Sex	Existence of metastases	Serum CEA (ng/ml)
1	66	M	+ (a)	6·0
2	73	M	−	1·8
3	66	F	−	1·5
4	71	M	−	1·6
5	50	F	−	1·6
6	59	F	−	1·5
7	72	M	−	1·3
8	78	F	+ (b)	3·7 (1982 Oct.)* 6·7 (1983 May)*
9	64	M	+ (c)	1·8
10	55	M	+ (d)	12·3 (1982 Oct.)* 17·1 (1982 Nov.)* 29·0 (1982 Dec.)*

(a) Left inguinal and peri-iliac lymph node metastases.
(b) Both inguinal and femoral lymph nodes, and widespread skin metastases.
(c) One small inguinal lymph node metastasis.
(d) Left inguinal lymph nodes, lung and widespread bone metastases.
* Serum CEA levels became gradually higher as metastases increased in number and size.

DISCUSSION

There has previously been no systemic marker for Paget's disease. Our previous histochemical study (Furue *et al.*, 1983) showed that CEA was positive in the metastatic lesions of Paget's disease as well as in Paget's disease of the skin. In this paper, we have shown that three patients with genital Paget's disease who had widespread metastases showed elevated serum CEA levels. In two patients the CEA levels increased as their metastatic lesions became more widespread. Patients who had only a small metastatic lesion in one lymph node or who had no apparent metastases showed normal levels. These results show that elevated serum CEA levels were associated with Paget's disease only when it showed widespread metastases. A preliminary study has shown that a tissue culture medium of Paget cells, which contained about 30% CEA-positive β_2 microglobulin-negative cells (presumably Paget cells), had high CEA level whereas that of normal epidermal cells showed less than 1 ng/ml CEA level (unpublished observation). This suggests that Paget cells produce and secrete CEA. There seems to be a relationship between tumour burden and serum CEA levels in Paget's disease and the serum CEA level in Paget's disease may be a useful systemic marker for metastasis.

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Pharmacology and Treatment

A randomized double-blind comparison of PUVA-etretinate and PUVA-placebo in the treatment of chronic plaque psoriasis

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SUMMARY

Twenty-eight patients with chronic plaque psoriasis affecting 20–40% of their body surface were treated with either PUVA and placebo (thirteen patients) or PUVA and etretinate (0·75 mg/kg) (fifteen patients). PUVA was given three times a week for a maximum of 10 weeks after a 2-week period on placebo or etretinate alone. Four patients failed to clear with PUVA and placebo compared with one patient with PUVA and etretinate, and the mean total UV-A dose to clear was lower with etretinate (mean = 62·1 J/cm^2) than with placebo (mean = 77·3 J/cm^2) but none of these differences were significant. Because the additional response to etretinate was only marginal, whilst unwanted effects were common, we found no advantage in adding etretinate to PUVA.

Photochemotherapy with 8-methoxypsoralen and UV-A (PUVA) is a reliable and effective method of treating chronic plaque psoriasis (CPP) (Parrish *et al.*, 1974; Rogers *et al.*, 1979b). Since the risk of long-term toxicity, particularly skin cancer, is still unknown, treatment should be kept to the minimum that is consistent with effectiveness.

It has been suggested that etretinate has a therapeutic effect on psoriasis whether used alone (Fredericksson & Pettersson, 1978; Lassus, 1980) or in combination with other treatments (Orfanos & Runne, 1976; Van Der Rhee *et al.*, 1980) including PUVA (Fritsch *et al.*, 1978; Michaelsson, Noren & Vahlquist, 1978; Heidbreder & Christophers, 1979; Lauharanta, Juvakoski & Lassus, 1981) and preliminary studies suggested that when etretinate was added to a PUVA regimen psoriasis cleared with a smaller dose of UV-A. We have now attempted to confirm this using objective measurements of response and a randomized controlled double-blind clinical study in as far as side-effects of etretinate made this possible.

METHODS

The trial was conducted on outpatients in the Skin Department who agreed to take part after the study was explained to them.

Patient selection and allocation

Thirty patients with CPP affecting 20–40% of the skin and thought to be suitable for treatment with PUVA were admitted to the trial and allocated at random to etretinate or placebo: one patient had previously failed to respond to PUVA. No patient had aphakia or abnormal renal or hepatic function, but those under 18 years of age and women of childbearing age (unless they agreed to use an effective method of contraception during the study and for 1 year thereafter) were excluded from entry. It was decided to restrict the study to patients of skin types II and III (Rogers *et al.*, 1979b) as the number of patients was relatively small and it was felt a more homogeneous population would be obtained this way with better matching between test and placebo groups.

Etretinate regimen

The daily dose of etretinate was approximately 0·75 mg/kg and was calculated from body weight as follows: less than 60 kg – 35 mg; 60 to 80 kg – 55 mg; more than 80 kg – 65 mg.

Etretinate or placebo tablets which were indistinguishable from one another were started 2 weeks before the PUVA, and were continued throughout the study.

PUVA regimen

8-Methoxypsoralen was given orally in a dose of 0·6 mg/kg body weight and 2 hours later UV-A was given, the initial and subsequent exposure time being determined as previously described (Rogers *et al.*, 1979b). The starting dose of UV-A calculated from skin type was subsequently increased only if the patient failed to respond, and burning was an indication for decreasing the dose or missing treatments. PUVA was continued for 10 weeks, or until clinical clearing was achieved, whichever was sooner.

Other treatment

With the exception of emulsifying ointment all topical and systemic therapy was stopped 4 weeks before entering the trial.

Assessment of response

Although assessment was carried out without knowledge of patient treatment allocation, characteristic side-effects made it obvious that some patients were taking etretinate.

Clinical. The total UV-A dose, number of treatments and time taken to achieve clearance were noted. Clearance was defined as less than 2% of the body surface being involved by plaque psoriasis (excluding the scalp). Those patients who had not cleared within 10 weeks of starting PUVA were considered to be treatment failures and etretinate or placebo was stopped.

Measurement. In the first twenty patients (ten PUVA-placebo and ten PUVA-etretinate) measurements of plaque thickness and evaporative water loss were taken once a week for the duration of treatment using the same forearm plaque throughout. Plaque thickness was measured with modified Harpenden calipers and evaporative water loss with a Servomed evaporimeter (Rogers *et al.*, 1979a).

Laboratory investigations

Blood count, serum bilirubin, alkaline phosphatase, aspartate aminotransferase and gamma glutamyl transferase were measured weekly. Serum urea and electrolytes, thyroid function tests,

TABLE 1. Clearance data

	Number of patients treated	Number of patients who cleared	Total UV-A dose (J/cm²) to clear Mean ± s.e.	Number of PUVA exposures to clear Mean ± s.e.	Duration of PUVA* treatment to clear (days) Mean ± s.e.
Etretinate	15	14	62·1 ± 9·0	17·1 ± 1·7	40·3 ± 4·3
Placebo	13	9	77·3 ± 14·8	18·2 ± 2·1	49·4 ± 6·7
		NS ($P > 0{\cdot}05$)	NS ($P > 0{\cdot}05$)	NS ($P > 0{\cdot}05$)	NS ($P > 0{\cdot}05$)

* Excludes 14-day pre-PUVA period.

fasting serum lipids and HDL cholesterol were measured and hand X-rays with step wedge for densitometry (Anderson, Shimmins & Smith, 1966) were taken at the beginning and end of treatment.

RESULTS

Two of the thirty patients failed to complete the trial: one patient developed an intercurrent illness unrelated to the psoriasis or its treatment and the other failed to attend.

Clinical response

One patient failed to clear after 10 weeks PUVA in the PUVA-etretinate group and four failed in the PUVA-placebo group ($P > 0{\cdot}05$, Fisher's exact test). The patient who had previously failed to respond to PUVA had been allocated at random to PUVA-placebo and he failed to clear. The time, total cumulative UV-A dose and the number of treatments required to achieve clearance are summarized in Table 1. There was no significant difference ($P > 0{\cdot}05$) between the two groups. For this analysis we excluded patients who failed to clear. The greater duration of treatment in the PUVA-placebo group compared with the PUVA-etretinate group despite virtually the same number of PUVA exposures is due to temporary withdrawal of treatment due to burning and missed treatments due to holidays.

Plaque thickness and evaporative water loss

The results are shown in Figs 1 and 2. Fig. 1 shows the change in plaque thickness over the 10-week trial period. The uninvolved paralesional skinfold thickness has been subtracted from the skinfold thickness of involved psoriatic plaque to give plaque thickness. Comparison of the slopes obtained by linear regression of all the data gave a gradient of $-0{\cdot}18$ for the etretinate group and $-0{\cdot}12$ for the placebo group. Fig. 2 shows the change in water evaporation rate with time: regression of all the data gave a gradient of $-1{\cdot}91$ for the etretinate group and $-1{\cdot}10$ for the placebo group.

Side-effects

Clinical (Table 2). Patients were asked to report on specific side-effects according to a check list given to them at the end of the trial. Many of the possible effects were scored by those on placebo, but hair loss was confined to the PUVA-etretinate group, dryness and cracking of the

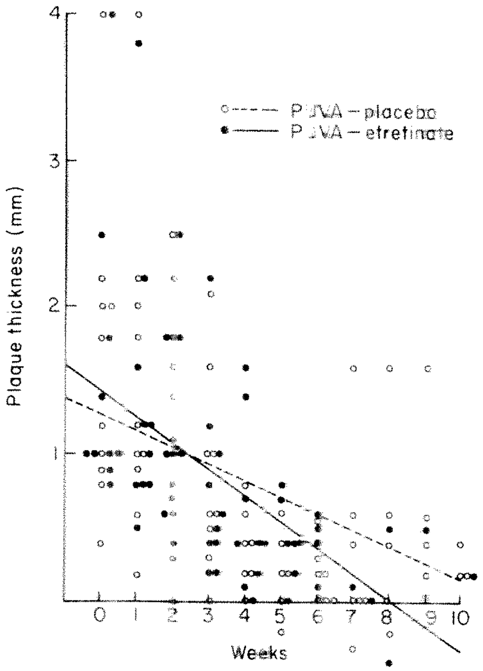

FIGURE 1. Reduction in plaque thickness with time. Plaque thickness was measured by subtracting the skinfold thickness of uninvolved paralesional skin from the skinfold thickness of a psoriatic plaque. The same forearm plaque was used for all measurements. PUVA-etretinate (n = 10), linear regression of this data gave a slope of −0·18 and intercept of 1·46. PUVA-placebo (n = 10), slope −0·12, intercept 1·3.

lips occurred more often and was worse in the PUVA-etretinate group. In only one patient on etretinate were the unwanted effects described as severe and in no case did side-effects necessitate stopping treatment.

Laboratory investigation. No abnormalities were found in any of the biochemical measurements nor in bone density measured with a step wedge.

DISCUSSION

Our findings show a small but insignificant difference in clinical response of CPP between PUVA-placebo and PUVA-etretinate. As with the clinical response there was no significant difference in the rate of response measured objectively by plaque thickness and transepidermal water loss, although both indices returned to normal faster in the etretinate group. Our findings contrast with those of Honigsmann & Wolff (1983) who did find a statistically significant difference between the doses of UV-A required to clear their two groups (106 ± 115 and 41 ± 36 J/cm²). Their study differed from ours in several respects: they studied more patients (133 in all) but did not allocate them at random or use placebo controls; they included patients of all skin types; they used 1 mg etretinate/kg; they gave PUVA four times a week compared with our three and their method of assessing starting dose was different. We can exclude the possibility that we gave more UV-A than necessary to the PUVA-etretinate group because their erythemal response was no greater than that of the placebo group. Another possibility is that Honigsmann & Wolff (1983) gave more PUVA than necessary to the patients who did not receive etretinate.

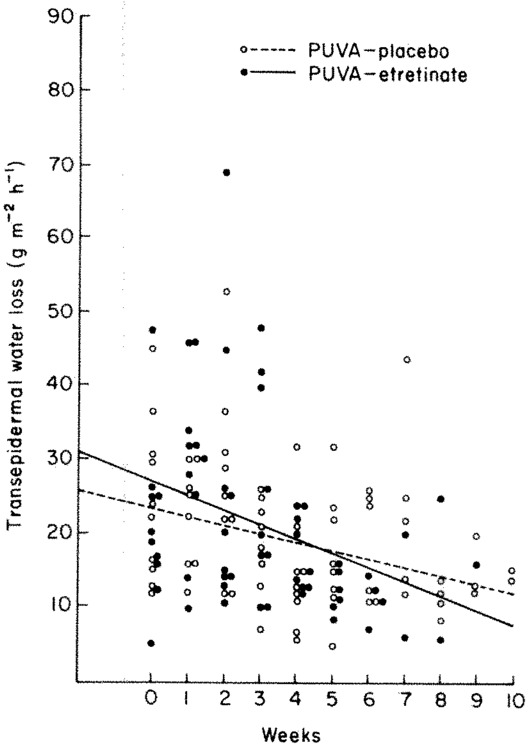

FIGURE 2. Reduction in water evaporation rate with time. Water evaporation rate measured over the same forearm plaque at weekly intervals in the PUVA-etretinate (n = 10, slope −1·91, intercept 26) and PUVA-placebo (n = 10, slope −1·09, intercept 23) treated patients.

TABLE 2. Clinical side-effects in all patients who completed the study

	Etretinate n = 15	Placebo n = 13
Dry cracked sore lips	13	7
Nausea	2	6
Dry mouth	5	6
Headaches	2	4
Hair loss	3	0
Itching	6	6
Palmoplantar desquamation	3	1
Nose bleeds	0	0
Sore eyes	2	2
Loss of appetite	1	1
Thirst	0	2
Paronychia	0	1
Sweating	3	3
Drowsiness	2	3

By contrast to CPP we have found a significant reduction of PUVA dosage with PUVA-etretinate compared with PUVA-placebo in palmoplantar pustular psoriasis (Lawrence *et al.*, 1984).

A high prevalence of side-effects occurred in the placebo group as well as with PUVA-etretinate, but hair loss occurred only with etretinate and cracking of the lips was more severe than with placebo. This difference in magnitude of unwanted effects was sufficient to invalidate the double-blind trial. Sore lips were relatively easily dealt with by emulsifying ointment. We did not find the abnormalities of serum lipids and liver function that others have found with etretinate (Ellis *et al.*, 1982) and that we have found with higher doses (1 mg/kg) of etretinate in palmoplantar pustular psoriasis (Lawrence *et al.*, 1984) and with isotretinoin (Lyons *et al.*, 1982; Marsden, Shuster & Laker, 1983). Whilst the unwanted effects were not difficult to manage, in the absence of an obvious beneficial effect our findings showed no advantage in adding etretinate to PUVA therapy of CPP.

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A comparison of PUVA-etretinate and PUVA-placebo for palmoplantar pustular psoriasis

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SUMMARY

Seventeen patients with palmoplantar pustular psoriasis and three with hyperkeratotic psoriasis of palms and soles were treated with either PUVA-etretinate (1 mg/kg) or PUVA-placebo. Patients were randomly allocated to each group and the trial was conducted according to a double-blind protocol, so far as the side-effects of etretinate made this possible. PUVA was given three times a week for a maximum of 18 weeks, after 2 weeks on daily placebo or etretinate alone. All ten patients in the PUVA-etretinate group cleared, but there were four failures in the PUVA-placebo group ($P = 0{\cdot}03$). The PUVA-etretinate treated patients required significantly fewer PUVA treatments (13·1 ± 2·9; mean ± s.e.) and cleared in a significantly shorter time (30·3 ± 7·1 days) than the PUVA-placebo group (23·2 ± 4·2 treatments; 59·2 ± 11·5 days, $P < 0{\cdot}05$). The cumulative UV-A dose to clear was less in the PUVA-etretinate group (53·9 ± 18·5 J/cm^2) than the PUVA-placebo group (113·1 ± 33·4 J/cm^2). This difference was not significant due to the exceptionally large dose of UV-A used on one patient but the results were significant when it was excluded.

The therapeutic advantage of adding etretinate to PUVA is offset by the side-effects of cheilitis, hair loss and peeling skin which occurred in eight of the ten PUVA-etretinate patients, and an increase in fasting triglyceride concentrations and serum alkaline phosphatase activity.

Palmoplantar pustular psoriasis (PPPP) responds poorly to topical treatment, but it does respond to photochemotherapy with psoralen and UV-A (PUVA) (Morison, Parrish & Fitzpatrick, 1978; Murray, Corbett & Warin, 1980) although the UV-A dose required may be considerable. Oral etretinate has also been used for PPPP (Jansen, Hollmen & Pajarre, 1979), but although there may be improvement few patients actually clear (Reymann, 1982) and an adequate therapeutic action is often limited by unacceptable side-effects. We therefore decided to see whether there was any advantage in combining etretinate with PUVA.

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METHODS

Patients

Seventeen patients with PPPP and three with hyperkeratotic psoriasis of palms and soles currently being treated in the Skin Department took part in the trial after its nature had been explained to them. Patients were selected mainly by their willingness to take part, but we excluded women of childbearing age unless they were using an effective contraceptive before, during and for at least 1 year after termination of treatment, and those aged less than 18 years. None had aphakia or abnormalities of renal or hepatic function. They had been attending hospital for 1–16 years (mean 6·25 years) and most had failed to respond to previous treatments including anthralin, coal tar and topical corticosteroids. The diagnosis was made on clinical grounds. In five patients only the soles were involved and in fifteen both the palms and soles; twelve patients had visible pustules at the start of the trial and pustule formation had occurred previously in all but three of the twenty patients. The remaining three had hyperkeratotic palm and sole psoriasis. Nine patients had classical psoriasis at other sites including the three with hyperkeratotic psoriasis.

Patients were randomly allocated to either PUVA plus placebo (PUVA-placebo) or PUVA plus etretinate (PUVA-etretinate), and the trial was conducted as if double-blind, although in most patients differences in side-effects and patient response made a double-blind assessment impossible. In the PUVA-placebo group (nine female, one male, mean age 52·2 ± 4·6 years, disease duration 9·3 ± 3·2 years) six patients had disease affecting the palms and soles and in four it was restricted to the soles; six patients had pustules at the start of the trial, three had previously been seen to develop pustules and one had hyperkeratotic psoriasis without pustule formation. In the PUVA-etretinate group (seven female, three male, mean age 60·6 ± 3·0 years, disease duration 16 ± 4·8 years) five patients had involvement of the palms and soles, and five patients had only sole involvement; five had visible pustules at the start of the trial, three gave a history of pustule formation and two had never had pustules. None of these differences were significant and, overall, groups were well matched for skin type and clinical disease severity.

All anti-psoriatic treatment was stopped 1 week before the trial, except emulsifying ointment which was allowed throughout.

Treatment regimens and measurements

The trial lasted a maximum of 20 weeks; for the first 2 weeks patients received only placebo or etretinate, and from week 3 to 20 they received PUVA-etretinate or PUVA-placebo.

PUVA

Oral 8-methoxypsoralen (0·6 mg/kg) was given 2 hours before phototherapy. UV-A was generated from a Waldmann 180 unit. The starting dose of UV-A was 1·5 J/cm^2 for skin type I, 2·5 J/cm^2 for skin type II, 3·5 J/cm^2 for skin type III and 4·5 J/cm^2 for skin type IV (Rogers *et al.*, 1979). UV-A dose was increased by 0·5–1 J/cm^2 after every three treatments if the rash was not improving. Burning was an indication for temporary withdrawal of PUVA or dose reduction. PUVA was given three times a week until the psoriasis had cleared: if it had not cleared after 18 weeks PUVA was stopped and the treatment was considered to have failed. Clearance was defined as the disappearance of all pustules with mild or absent scaling and erythema.

Etretinate

Etretinate or placebo was administered as identical capsules in a single daily dose, correspond-

TABLE 1. Clearance data

	Number of patients treated	Number of patients who cleared	Total UV-A dose to clear $(\mathrm{J/cm^2})$, mean ± s.e.	Number of treatments to clear, mean ± s.e.	Duration of PUVA treatment to clear (days), mean ± s.e.
PUVA-etretinate	10	10	$53{\cdot}9 \pm 18{\cdot}5$	$13{\cdot}1 \pm 2{\cdot}9$	$30{\cdot}3 \pm 7{\cdot}1$
PUVA-placebo	9	5	$113{\cdot}1 \pm 33{\cdot}4$	$23{\cdot}2 \pm 4{\cdot}2$	$59{\cdot}2 \pm 11{\cdot}5$
		$P=0{\cdot}03$ Fisher's Exact test	$P>0{\cdot}05$* NS	$P<0{\cdot}05$*	$P<0{\cdot}05$*

Figures show mean ± s.e.m.
* Wilcoxon rank-sum test.

ing to approximately 1 mg/kg etretinate according to body weight: <56 kg 50 mg, 56–65 kg 60 mg, 66–74 kg 70 mg, >75 kg 80 mg, starting 14 days before PUVA therapy.

Disease assessment

Clearing. Before the trial and at weeks 10 and 20, or at clearance of the rash, disease severity was assessed by the total number of new pustules and the extent of scaling and erythema scored as severe, moderate, mild, none (3–0). On clearing or at the end of the trial the cumulative UV-A dose, number of treatments and duration of therapy were calculated.

Follow-up after stopping treatment. Patients were sent a questionnaire 24 weeks after finishing treatment and asked to report the length of clinical remission.

Side-effects

At each visit patients were asked if they had noticed any adverse effects.

Investigations

Measurements of the following were made before and at the end of therapy: fasting cholesterol and triglyceride, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, bilirubin, urea and electrolytes, and full blood count. Results were analysed by Fisher's exact test and the Wilcoxon rank-sum test.

RESULTS

Response

Nineteen patients completed the study. One patient, who was receiving placebo, was withdrawn during the sixth week of the study because she developed acute diverticulitis, presumably unrelated to treatment. At the time she still had pustules with severe scaling and erythema. The three patients with hyperkeratotic psoriasis all cleared: one had received PUVA-placebo and two PUVA-etretinate. All patients in the PUVA-etretinate group cleared, compared with four who had still not cleared after 18 weeks' PUVA treatment in the PUVA-placebo group ($P = 0\cdot03$, Fisher's exact test). The duration of treatment was significantly shorter ($P < 0\cdot05$), and the number of treatments required to clear the rash was significantly less ($P < 0\cdot05$) in the PUVA-etretinate group compared to the PUVA-placebo group. The cumulative dose of UV-A required to clear the rash was considerably less in the PUVA-etretinate group, but this difference just failed to achieve statistical significance ($P > 0\cdot05$). When the values from one patient in the PUVA-etretinate group who required 204 J/cm^2 to clear (a dose more than 2 SDs outside the calculated mean) were excluded there was a significant difference between the groups ($P < 0\cdot05$ Student's t-test) in the cumulative UV-A dose to clear (PUVA-etretinate $37\cdot22 \pm 8\cdot9$, mean ± s.e.).

Pustule counts fell to zero by week 20 or earlier in all five patients in the PUVA-etretinate group who had pustules at the start of the trial. In the PUVA-placebo group pustule counts fell to zero in three of the six patients with pustules, but three others still had multiple pustules at the end of the trial.

Erythema and scale scores fell by week 20 or earlier in nine of the ten patients in the PUVA-etretinate group and six of the nine in the PUVA-placebo group ($P > 0\cdot05$, Fisher's exact test).

Relapse after treatment

Follow-up replies were received from all the fifteen patients who cleared. In the PUVA-etretinate group two patients remained clear after 6 months, the other eight remained clear for an average of 9 weeks. In the PUVA-placebo group two remained clear after 6 months and the other three remained clear for an average of 12 weeks ($P > 0\cdot05$).

Side-effects

Side-effects were encountered in eight of the patients receiving PUVA-etretinate but in none of those in the PUVA-placebo group. Six patients had cheilitis, which was severe in three, moderate in one and mild in two. It developed in the first week in four patients. Four had severe diffuse scalp hair loss, which came on between 5 and 9 weeks after starting etretinate, and continued for 2–8 weeks. In three patients it occurred after the treatment was stopped at the end of the trial. Regrowth was eventually complete in all patients. Two patients complained of peeling of the skin on the palms and soles, and in one patient this became generalized on the body and was associated with pruritus. She later developed nail softening. Another patient complained of dryness of the nasal mucosa which oozed blood.

Fasting triglyceride concentrations increased significantly ($P < 0\cdot05$ Student's t-test) in the PUVA-etretinate group from a pretreatment concentration of $1\cdot5 \pm 0\cdot31$ (normal range $0\cdot45$–$1\cdot8$) mmol/l to $2\cdot05 \pm 0\cdot46$ (mean ± SE) mmol/l after treatment. In the PUVA-placebo group triglyceride concentrations were $1\cdot58 \pm 0\cdot18$ mmol/l pretreatment and $1\cdot64 \pm 0\cdot36$ mmol/l post-treatment. Fasting cholesterol concentrations were unchanged in the PUVA-etretinate group, with a pretreatment concentration of $6\cdot2 \pm 0\cdot48$ mmol/l and post-treatment concentration of $6\cdot3 \pm 0\cdot42$ mmol/l.

In the PUVA-etretinate group there was a significant increase ($P < 0\cdot05$) in the serum alkaline phosphatase activity, from a pretreatment concentration of 107 ± 11 (normal range 30–130) u/l to 120 ± 11 u/l post-treatment. The alkaline phosphatase was not typed, but other liver enzymes showed a small increase in the activity in this group. γ-glutamyl transferase activity rose from

19·8 ± 4·5 (normal range women < 35, men < 60) u/l to 24·8 ± 5·9 u/l ($P > 0·05$) post-treatment, and aspartate aminotransferase activity increased from 20·3 ± 3·2 (normal range < 37) u/l to 27·4 ± 3·7 u/l ($P > 0·05$). These changes were not found in the PUVA-placebo group. There was no significant change in serum bilirubin, urea and electrolytes or the blood count in either group.

DISCUSSION

We found the addition of etretinate to PUVA to be superior to PUVA alone for the treatment of PPPP, in that more patients cleared and the response was faster, requiring a smaller cumulative dose of PUVA. Thus, our findings suggest that adding etretinate to PUVA has a greater effect in PPPP than in chronic plaque psoriasis (CPP) (Parker *et al.*, 1984) in which we found a slight and insignificant decrease in the cumulative dose, total treatment time and number of treatments with PUVA, although we did use a smaller dose of etretinate. Fritsch *et al.* (1978) found a similar response in adding etretinate to PUVA in palmoplantar psoriasis, their patients responding after a mean of seven treatments. However, theirs was an open study of twelve patients who were not allocated at random and not compared directly with a similar control group: their PUVA and PUVA-etretinate regimens were different from ours, and it is not clear if their patients had PPPP or hyperkeratotic psoriasis.

It is not clear what clinical types were included in other previous studies of psoriasis of palms and soles. Our observations apply mainly to pustular psoriasis (seventeen out of twenty). Only three had hyperkeratotic psoriasis and, although their response was similar, the numbers are too small for comparisons.

The unwanted side-effects of etretinate were distressing and occurred in eight out of the ten patients receiving the drug. Hair loss occurred in 40% of the patients in this study compared with 20% of the patients receiving etretinate for CPP (Parker *et al.*, 1984); the etretinate dose was 0·75 mg/kg in the latter study which suggests that this side-effect is dose dependent. Whilst this is completely reversible it can be very distressing. Alopecia and the early appearance of severe cheilitis in six of our patients invalidated the double-blind design of the trial. This is a serious defect both of the present and past studies of etretinate. To overcome this in future studies, clinical assessment should be made without access to history or appearance of face and scalp. As with isotretinoin (Lyons *et al.*, 1982; Marsden *et al.*, 1983) there was a significant increase in fasting triglyceride concentration with etretinate similar to that found by Ellis *et al.* (1982). This may be dose-related, as we did not find it with 0·75 mg/kg etretinate in CPP (Parker *et al.*, 1984). Alkaline phosphatase activity also rose significantly, but no isoenzyme analysis was done so we cannot establish whether this was of hepatic, intestinal or bone origin, although there was also a small increase in γ-glutamyl transferase and aspartate amino transferase activity, suggesting that this alkaline phosphatase activity was of hepatic origin.

Whilst the present trial demonstrates that more patients with PPPP respond, and in a shorter time and with fewer PUVA treatments and total dosage with PUVA-etretinate than with PUVA alone, it is not yet clear whether these advantages outweigh the unwanted effects of etretinate. For these reasons it would be helpful to establish whether a similar therapeutic effect could be obtained using a lower and less toxic dose of etretinate. The addition of etretinate should, however, prove useful in the treatment of patients with PPPP resistant to PUVA alone.

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Case Reports

Localized heat urticaria associated with a decrease in serum complement factor B (C_3 proactivator)

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SUMMARY

A case of localized heat urticaria is reported in a 51-year-old woman who within a few minutes of contact with warm water developed erythema and swelling sharply localized to the heated area. After a hot bath urticarial lesions appeared over large areas of her body, accompanied by a feeling of weakness, but no other systemic symptoms. After challenge with heat by immersing her left arm in water heated to 42°C, a rapid decrease of her serum complement level of factor B was demonstrated, suggesting that activation of an alternative complement pathway plays a role in this form of urticaria. Biopsies for immunofluorescent study of complement and immunoglobulins were negative at 30 and 180 min after heat challenge. The dermal fibres and endothelial cells of dermal vessels were capable, *in vitro*, of complement binding before and after exposure to heat.

There are two distinct forms of heat urticaria. The more common is the generalized form in which pruritic weals develop after a rise in body temperature due to overheating (Hopkins, Kesten & Hazel, 1938; Jorizzo & Smith, 1982; Soter, 1981). The other form is localized heat urticaria which is very rare. It is produced by direct contact of the skin with heat. The urticarial lesions develop within a few minutes after heat exposure and are localized to the area of contact (Atkins & Zweiman, 1981; Daman *et al.*, 1978; Delorme, 1969; Greaves *et al.*, 1974; Leigh & Ramsay, 1975; Wise *et al.*, 1978; Starr, Brasher & Hammerschmidt, 1980; de Moragas, Gimenez-Camarasa & Noguera, 1973). In localized heat urticaria, systemic symptoms may occur if large areas of the skin are affected. Nausea, headache, dizziness and loss of consciousness have been described after a hot bath (Delorme, 1969). In addition, a familial form of localized heat urticaria has been reported in one family, where the lesions appear 1·5 to 2 h after heat exposure and last for 12 to 14 h (Michaelsson & Ros, 1971).

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We describe a patient with localized heat urticaria in whom decreased serum levels of complement factor B followed heat challenge, whereas in the immunofluorescent examinations no deposits of complement or immunoglobulins were found in the exposed area of the skin.

CASE REPORT

A 51-year-old waitress had a 5-year history of urticaria or angio-oedema after exposure to heat. The symptoms were provoked by steam released from the oven she used in her work and appeared mostly on her face and behind the ears. Also after a hot bath and sauna she experienced itching; wealing appeared on large areas of her body accompanied by a feeling of weakness but no other systemic symptoms. The lesions developed within a few minutes and disappeared later the same day, although occasionally the facial oedema lasted for 3 to 4 days. The patient herself had no past history of urticaria or atopy but her brother and grandmother had asthma. Except for mild hypertension (treated with diuretics) the patient was healthy. She was first admitted to hospital in February of 1980 because of an acute onset of urticaria after a hot bath. The following laboratory tests were found to be within normal limits: haemoglobin, red and white blood cell counts, leukocyte differential count, erythrocyte sedimentation rate and serum immunoglobulins (IgG, IgA, IgM and IgE). No antinuclear antibodies, rheumatoid factors, cryoglobulin or cold agglutinins were found in the patient's serum. Serum aliquots for C1 esterase, C3, C4 and CH50 yielded normal values before and after challenge with hot water. Intradermal tests to common allergens revealed no positive reactions. Physical exercise with a bicycle did not provoke urticaria. Intradermal metacholine skin test gave a negative result and intradermal injection of atropine did not block the urticarial reaction. There was no dermographism on firm stroking of the skin.

Challenge tests were performed in which the patient's left arm was immersed in water for 5 to 10 min. When her arm was immersed in water heated to 42°C a sharply demarcated erythema and slight swelling appeared on the contact area. When the temperature of the water was gradually lowered to 37°C, an erythema still developed, whereas water at 36°C did not provoke any reaction. The reaction began with itching and within 5 min sharply demarcated redness and slight swelling was seen corresponding to the water line. After 20 min the swelling was maximal and at that time the patient experienced a slight stiffening of the entire arm. One hour after the exposure was started, there was still itching and redness, but 2 h later the symptoms had disappeared. Repeated exposure to warm water heated to 37–42°C did not prevent subsequent urticarial response.

In March 1982 the patient was admitted to hospital for special studies. She was found to be in good health and the laboratory tests mentioned previously again gave normal results. She had been treated with an antihistamine (cinnarizin) which had been of some help. During this time she had carefully avoided hot baths and saunas and had not been in contact with hot steam at work.

Special investigations

The patient was again challenged to heat by immersing her left arm in water maintained at 37°C for 5 min. The arm was submerged in water up to the upper half of the upper arm and blood samples were collected from the exposed arm and contralateral arm prior to, as well as 5, 10, 15, 30 and 45 min after discontinuation of the heat exposure. The quantitative level of complement factor B was measured from serum samples by single radial immunodiffusion. The phenotype of factor B was FS-detected by agarose electrophoresis and immunofixation (kindly performed by

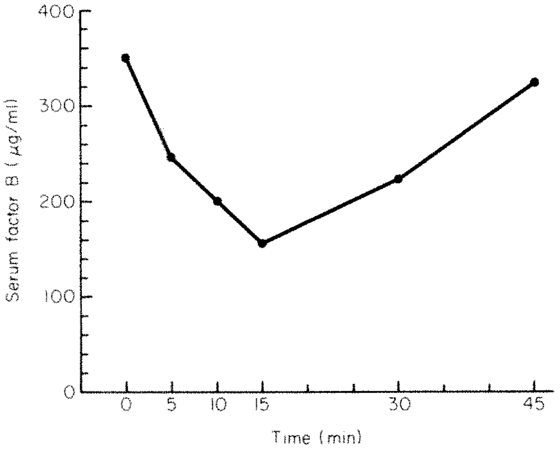

FIGURE 1. The rapid decrease of serum complement factor B value after heat challenge. A similar result was obtained after a repeated test.

M.-L.Lokki, M.Sc., Finnish Red Cross Blood Transfusion Service). Antiserum–agarose plates containing 3% agarose (Indubiose®) and goat anti-human Properdin factor B serum (Atlantic antibodies) in barbiturate buffer were used. Ten-microlitre samples of serum diluted 1:2 in barbiturate buffer were incubated on plates for 3 days at 4°C. The size of the precipitates was compared with known control serum (pooled human serum of healthy blood donors) and the amount of factor B was extrapolated from a standard curve. The factor B level was found to decrease from 346 μg/ml to 167 μg/ml within 15 min after discontinuation of the heat exposure (Fig. 1). After 30 min the level was already rising and reached the normal level 45 min after the heat challenge. From the contralateral arm the curve of the serum factor B level showed the same tendency but the decrease was not so great as with the exposed arm.

An age- and sex-matched healthy control person was investigated simultaneously and no decrease in the serum factor B level was observed.

Skin biopsies were taken from the inner arm (using 1% lignocaine anaesthesia) before and 30 and 180 min after exposure to heat. The specimens were cut and stained with haematoxylin and eosin, toluidine and Alcian blue, for routine light microscopy.

Perivascular round cell infiltration and swelling of the vascular wall were seen in specimens obtained 30 min after heat challenge. All peri-endothelial mast cells were intact.

Specimens for immunofluorescent examination of the skin were taken 0, 30 and 180 min after heat exposure. They were snap-frozen, and unfixed cryostat-cut sections were examined for *in vivo* bound immunoglobulins (IgG, IgA, IgM), complement (C1q, C3c) and fibrin, using FITC-conjugated antisera diluted 1:20 to 1:40 (Behringwerke, Marburg, F.R.G.). Concomitant sections were fixed with 3·5% paraformaldehyde, incubated with 1:10 diluted normal human serum and examined with FITC-conjugated anti-C3c antisera for an *in vitro* complement-binding capacity of epidermal and dermal structures (Linder, 1982). No immunoglobulin or complement deposits *in vivo* were found either in the epidermis or dermis before or after exposure to heat. The only pathological finding was the occurrence of granular fibrin deposits around but not within upper dermal vessels in 180-min biopsy specimens. *In vitro* examination revealed that complement bound to the outer epidermal layers, to subepidermal fibres and to endothelial cells of vessel walls in 0-, 30- and 180-min biopsy specimens, a finding corresponding to the result in normal skin.

DISCUSSION

Localized heat urticaria is rare and fewer than twenty cases have been reported. Most of these have been women (Atkins & Zweiman, 1981; Daman *et al.*, 1978; Delorme, 1969; Jorizzo & Smith, 1982). In most of the reported cases immediate wealing has taken place 5 to 20 min after the heat exposure. Systemic symptoms have been documented in about half of the cases (Delorme, 1969; Jorizzo & Smith, 1982).

Attempts to transfer skin reactivity to heat, using patients' sera (Prausnitz–Kustner) have failed (Greaves *et al.*, 1974). Repeated exposure to heat did not prevent subsequent urticarial response in our patient, a finding also reported by other investigators (de Moragas *et al.*, 1973; Daman *et al.*, 1978). Nevertheless, Leigh & Ramsay (1975) reported that tolerance to heat could be induced within 10 days after starting repeated challenge to hot water. No follow-up of their patient was reported.

The pathophysiology of this type of heat urticaria is not understood. Some studies suggest that the abnormal complement system plays a causative role (Daman *et al.*, 1978) while others prefer to look at it as a mediator release phenomenon (Atkins & Zweiman, 1981; Greaves *et al.*, 1974; Grant *et al.*, 1981). In our study the remarkably rapid decrease of the serum complement level of factor B provides evidence for activation of the alternative complement pathway and consumption of factor B during the heat exposure. Factor B, C3 proactivator, is the precursor of the key enzyme of the alternative pathway. The activation of this pathway is non-specific; it does not need immunoglobulin. It can be activated by a number of agents but the common denominator is still unknown. Differences in the factor B molecule may exist in regard to sensitivity to activation. Functional studies have shown defective variants of antigenically normal factor B molecule (Hauptmann, Tongio & Mayer, 1976). In our case no functional studies were performed, but our patient had a factor B phenotype which is common in the Finnish population (unpublished data).

Activation of C3 by factor B produces protein fragments that function as ligands between cells or as activators of cellular functions. C3a and C5a are hormone-like messengers (Hugli & Müller-Eberhard, 1978) which release histamine from mast cells and basophils, and hydrolytic enzymes from polymorphonuclear leukocytes (Ward & Newman, 1969; Goldstein & Weissmann, 1974). Both of these polypeptides induce an increase in vascular permeability when injected intradermally in very minute amounts (Vallota & Müller-Eberhard, 1973). In our patient endothelial swelling was evident in the 30-min biopsy specimen. Possibly due to vascular leakage, a perivascular fibrin deposition occurred in the 180-min biopsy specimen. However, no evidence of local complement participation was found by either *in vivo* or *in vitro* examinations. No complement deposits were found in skin specimens taken after exposure to heat, and in agreement with this, dermal fibres and endothelial cells of dermal vessels were capable of complement binding *in vitro*, both before and after exposure to heat.

The present study therefore suggests that alternative complement pathway activation is involved in the localized form of heat urticaria.

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Tick bite: two cases studied by scanning electron microscopy

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SUMMARY

The attachment of ticks to human skin has been studied by scanning electron microscopy. Intact specimens of *Ixodes ovatus* and *Ixodes persulcatus* were examined, and we also studied the skin of two patients who had been bitten by these two species. In the first case, the remains of the tick were visible and a homogeneous cement-like substance was observed on the dorsal hypostome and in the dermis, suggesting that the tick attaches itself to the host skin by a secretion. In the second case, the apices of some denticles of the hypostome were chipped. Two months later, the skin which the tick had attacked was biopsied and yellowish-brown particles, probably derived from the denticles, were seen in foreign body giant cells in the dermis.

Although ticks are vectors for some important human diseases, their method of attachment to skin has not to our knowledge been previously described. We have now studied two cases of tick bite due to *Ixodes ovatus* and *Ixodes persulcatus* respectively. The area of skin where the tick had been attached, and the parasite itself, were observed by light microscopy and by scanning electron microscopy. Intact examples of the same species of ticks were also examined. Some points of interest resulting from the scanning electron microscopic observation, including the secretion from the tick and changes in the tick's appearance, are discussed.

CASE REPORTS

Case 1. A 14-year-old Japanese girl was brought to the clinic after being bitten by a tick. The day before she came to the clinic, her mother had removed the tick with forceps. A purple-red lesion was present just below the patient's right clavicle, and in the centre of the lesion small black particles, the remains of the tick, were still present. The lesion was resected, and the tick was identified as an adult female *Ixodes ovatus*.

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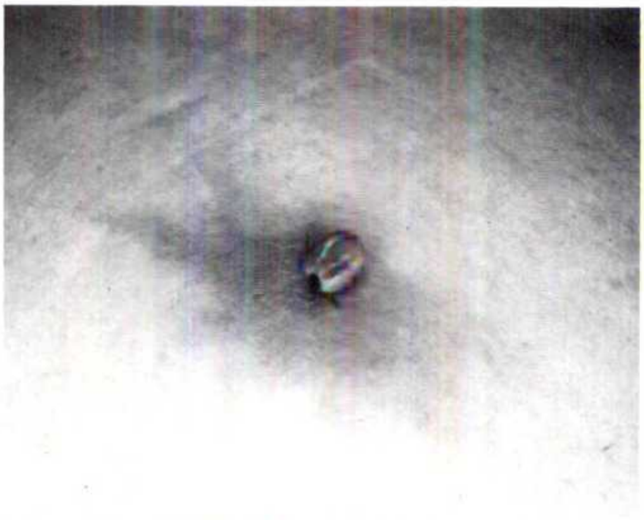

FIGURE 1. Case 2. The live tick was present in the centre of a red lesion.

Case 2. A 63-year-old Japanese female noticed that a small foreign body had attached itself to her back, and she gradually began to feel some pain in the back as it enlarged. A red lesion was present on her back on the right side, in the centre of which a live tick, approximately 5 mm in diameter, was present (Fig. 1). A small incision was made, and the tick was removed. When the lesion was observed with a magnifying glass, there appeared to be no part of the tick remaining in the patient' skin. The tick was identified as an adult female *Ixodes persulcatus*.

METHODS

The area of skin where the tick had been attached (case 1), the tick which had been removed (case 2) and two intact ticks (for comparison), were all fixed in 2·5% glutaraldehyde buffered with phosphate (pH 7·2) overnight and then washed again. The skin of the lesion was cut with a sharp thin knife approximately vertically, and this made the remains of the tick clearly visible. The materials were dehydrated with ethanol, and dried in a critical point dryer. They were then observed with a scanning electron microscope (JSM35C or HHS 2R). The area of skin attacked by the *Ixodes persulcatus* was re-examined 2 months after removing the tick, resected, fixed in 10% formalin, dehydrated in ethanol, stained with haematoxylin and eosin, and with Verhoeff-von Gieson.

RESULTS

Case 1. When the area of skin where the tick had attached itself was observed by scanning electron microscopy (Fig. 2), the epidermis between the skin surface and dermis was clearly visible as a thick line. In the dermis many collagen bundles were found. As for the remains of the tick on the skin surface, parts of the capitulum and right pedipalp were visible on the dorsal view. In the skin, the hypostome and the chelicerae had been inserted almost vertically into the dermis through the epidermis above. A homogeneous material, indicated by an arrow, was present under the chelicerae on the upper dorsal surface of the hypostome. This material

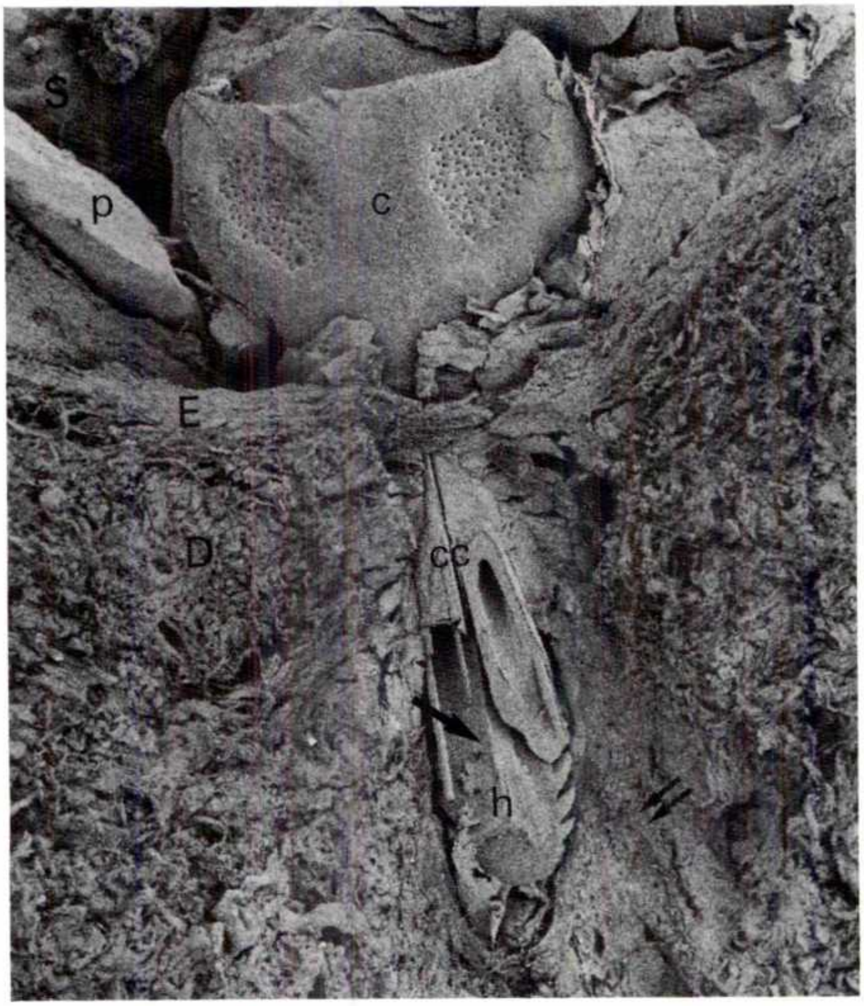

FIGURE 2. Case 1. The chelicerae (cc) and hypostome (h), emerging from the capitulum (c), had been inserted through the epidermis (E) into the dermis (D). A homogeneous material, indicated by an arrow, was present on the dorsal surface of the hypostome and continued into the dermis. Around the hypostome collagen tissues had lost their bundles, indicated by two arrows. S = skin surface, p = the right pedipalp (× 280).

continued into the dermis and collected in the cavity below the hypostome to form a solid mass. Around the hypostome, collagen tissues lost their bundles and became homogeneous (indicated by two arrows in Fig. 2). The denticles on the hypostome were found to be pointing backward in relation to the direction of insertion. A cement-like substance was seen on the hypostome (Fig. 3).

A ventral view of an intact *Ixodes ovatus* (adult female) was observed for comparison. The capitulum, body, and four pairs of legs were clearly visible (Fig. 4). The part of the tick which is inserted into the host skin was examined from dorsal view (Fig. 5). The chelicerae, the hypostome and the right pedipalp were found to emerge from the capitulum.

Case 2. The tick which had attached itself to the patient's back was examined under scanning

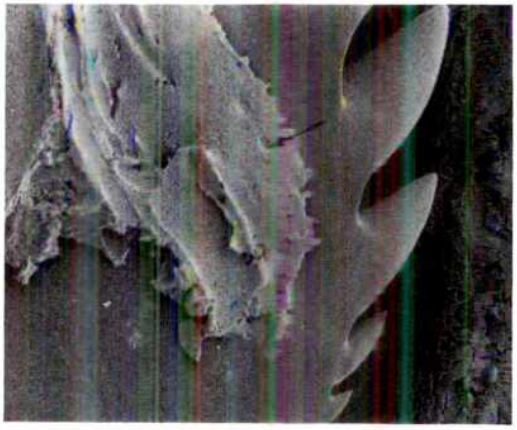

FIGURE 3. The homogeneous material, indicated by the arrow, was present on the dorsal surface of the hypostome. Slightly indented denticles were found to be pointing backward (× 1470).

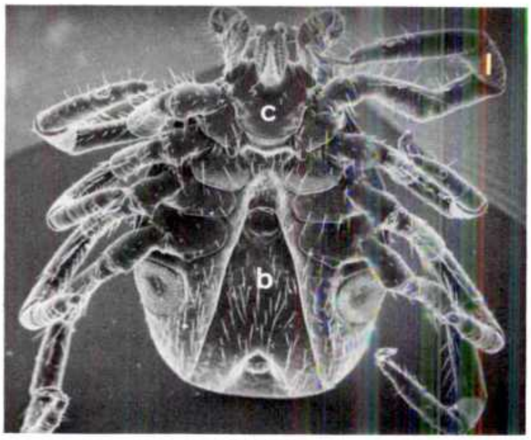

FIGURE 4

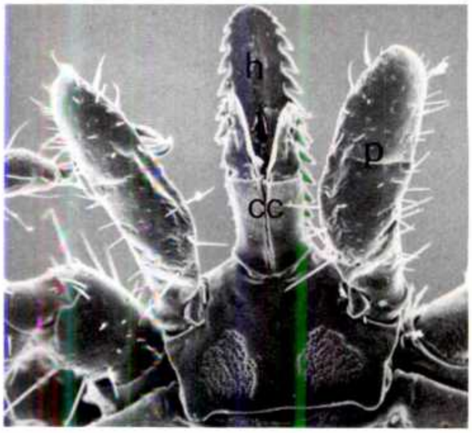

FIGURE 5

FIGURE 4. (Left) A ventral view of an adult female *Ixodes ovatus*. c = capitulum, b = body, l = four pairs of legs (× 50).

FIGURE 5. (Right) The part of the tick that is inserted into the skin, seen from the rear dorsal view. c = capitulum, cc = chelicerae, h = hypostome, p = right pedipalp (× 140).

electron microscopy. The part of the tick which is inserted in the host skin was examined from the ventral view. On the ventral surface of the hypostome a number of denticles appeared as a row of lines. The apices of some of these denticles, indicated by arrows, appeared to have been chipped, and were missing (Fig. 6).

The area of skin which had been attacked by the tick was observed by light microscopy 2 months after the tick had been removed. In the dermis, a homogeneous area with no elastic fibres was observed from the upper to the lower dermis. Patchy cellular infiltrations were observed (Fig. 7). High magnification views showed perivascular and periadnexal cellular infiltrations mainly consisting of plasma cells and lymphocytes. Foreign-body giant cells were associated with them. Yellowish-brown particles, indicated by arrows, were observed in the foreign-body giant cells (Fig. 8).

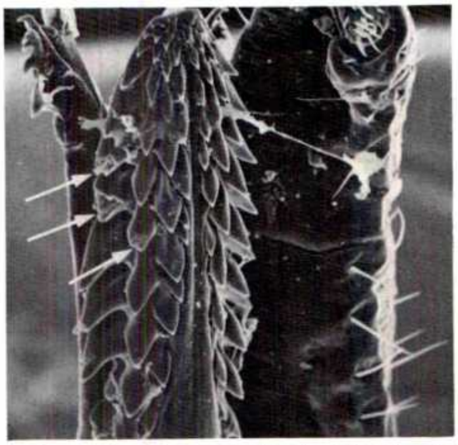

FIGURE 6. Case 2. The apices of some denticles appeared to be chipped and missing, indicated by arrows (× 210).

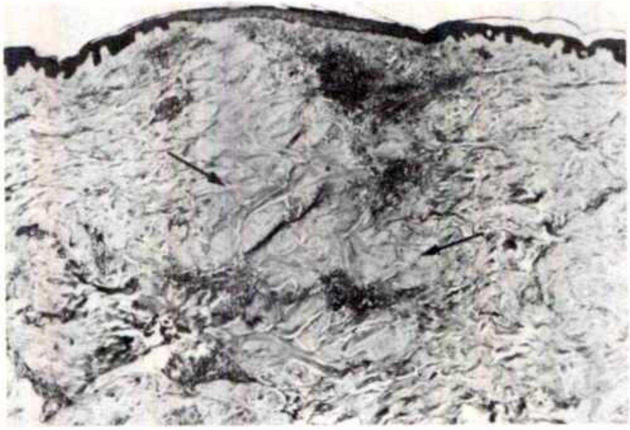

FIGURE 7. Two months after the tick had been removed, a homogeneous area was found to be present from the upper to the lower dermis, indicated by arrows (H & E).

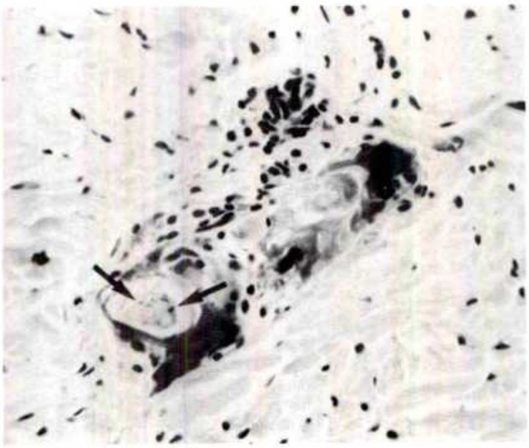

FIGURE 8. Yellowish-brown particles, indicated by arrows, were observed in the foreign-body giant cells (H & E).

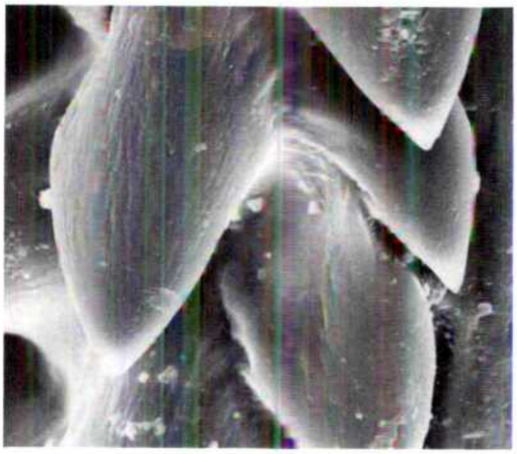

FIGURE 9. The denticles of an adult female *Ixodes persulcatus* (× 1440).

When the intact *Ixodes persulcatus* was observed under scanning electron microscopy, the denticles on the hypostome were slightly waved, and their apices were found to be remarkably sharp (Fig. 9).

DISCUSSION

This appears to be the first report to present clear scanning electron microscopic photographs of the attachment of a tick to the host skin, and the changes that can occur after removal of the tick from the host skin, although a recent report has studied the intact *Ixodes* by scanning electron microscopy (Ryckmanns & Wolff, 1981).

In the first case, a homogeneous cement-like substance was observed under scanning electron microscopy on the dorsal hypostome and continuing into the dermis. Because of its location and form, this homogeneous material would seem to be a secretion from the tick. It has been suggested that this secretion is from the salivary gland of the tick (McSwain, Essenberg & Sauer, 1982). Furthermore, collagen bundles could not be detected around the hypostome. Similar findings were also observed in case 2. The homogeneous tissue seems to represent a liquefaction of the tissue under the influence of some secretion from the tick (Binnington & Kemp, 1980). This liquefaction has been found up to 2 months after removal of the tick, as observed in case 2. The denticles on the hypostome make it difficult to remove the tick once the hypostome has been inserted (Patterson, Fitzwater & Connell, 1979).

In the second case, scanning electron microscopy showed that the apices of some of the denticles on the hypostome had been chipped and were missing. Two months after the tick had been removed, yellowish-brown particles could be observed in the giant cells of the dermal cellular infiltrations under light microscopy. A previous report also stated that under light microscopy the part of the tick's body which was inserted into the host skin appeared yellowish-brown (Takino, 1978). Since both studies used the same haematoxylin and eosin staining technique, and since the colour of the hypostome in the previous report and the particles in the present report are almost identical, it seems quite likely that the chipped particles of the denticles were ingested into giant cells.

In the case of tick bites, the normal period of attachment is 4–14 days. Treatment may consist

TABLE 1. Some tick-borne diseases

Viral diseases:
  Colorado tick fever, Crimean-Congo haemorrhagic fever, Russian spring–summer encephalitis, tick-borne encephalitis, Omsk haemorrhagic fever, Kyasanur forest disease, Powassan encephalitis.
Rickettsial diseases:
  Rocky Mountain spotted fever, Siberian tick typhus, Queensland tick typhus.
Bacterial diseases:
  Tick-borne relapsing fever, American relapsing fever, African relapsing fever, Asian relapsing fever.
Protozoal disease:
  Human babesiosis.
Other diseases:
  Lyme disease, tick paralysis, Q fever, tularaemia.

of application of chloroform, ether, paraffin, etc. to induce detachment, merely waiting for the tick to detach naturally (Sakai *et al.*, 1976) or even resection of the lesion. The results of the present study show that attempts to remove the living tick by force may result in pieces of tick being left in the skin. Resection of the entire lesion would seem to be a useful means of preventing related infections and the punch biopsy method would seem to be effective for ticks that cannot be removed by other means (Patterson *et al.*, 1979). Risk of human infection can be reduced by prompt removal of attached ticks (Piesman & Spielman, 1980). Tick infestation may cause a variety of skin diseases including: granuloma (Tobias, 1949), lymphoma (Gottron & Schonfeld, 1960), erythema chronicum migrans (Scrimenti, 1970), acrodermatitis chronica atrophicans (Goor & Schwarz-Speck, 1972), pustules with a red halo (Fujiwara, Ono & Kawashima, 1981) and blisters (Hoogstraal & Gallagher, 1982).

Table 1 provides a list of some non-cutaneous tick-borne diseases. The discovery of a new fastidious mycoplasma in ticks offers opportunities to explore the possible role of these agents in human diseases (Tully *et al.*, 1981).

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Comment

ON CELL KINETICS IN PSORIASIS

A current view of psoriasis is that the normal events of cell maturation fail to reach completion because they are not given enough time, or in other words that the maturation rate cannot adjust itself to the increased turnover rate of the tissue (Farber & Van Scott, 1979). However, several facts (Baker & Wilkinson, 1979) suggest the other possibility that an alteration, rather than a deficiency, in epidermal maturation is the primary cause of the typical psoriatic morphology.

(1) In lamellar ichthyosis, where the proliferation rate is higher than normal (Frost *et al.*, 1966; Heenen & Galand, 1984), there is formation of a thickened granular layer.

(2) During psoriasis treatment, there is formation of a granular layer before the reversal of proliferation activity (Fry & McMinn, 1968).

(3) In experimentally-induced acceleration of turnover rate, cell maturation proceeds normally (Christophers & Braun Falco, 1970).

(4) In psoriatic lesions, tonofilaments are sparsely formed in basal cells (Brody, 1962; Lagerholm, 1965) supporting the contention that keratinization is already disturbed in its earliest stages.

Histologically (Fig. 1), the stable psoriatic plaque is characterized by: (1) an increased size of the germinative and differentiated compartment; (2) a higher proportion of germinative cells (Mali, 1979: Heenen & Pennequin, unpublished results). The turnover times of the germinative compartment and of the differentiated cells are drastically reduced (Wright, 1980). The kinetic parameters reflect the *equilibrium* reached in the stable clinical phase of the lesions, with cell production from the germinative layers compensating for cell loss by desquamation. However, these parameters do not account for the *ontogenesis* of the lesion. An increased turnover rate (by reduction of cell cycle time or increase of growth fraction) cannot induce the typical psoriatic morphology and particularly the increased size of the germinative compartment (Fig. 2). Let us consider the case of a cell cycle time decreasing immediately by one half. The rate of cell production of the germinative compartment will thus double and as a consequence the flux of cells in the differentiated compartment will also double. For a time equal to the transit time (until the flux of cells has doubled throughout the compartment) the differentiated compartment will grow. Then a new steady state will be installed and in this example the proportion of germinative cells will *decrease* proportionately to the cell cycle time, not increase as in the case of psoriasis.

Let us consider how these modifications can be accounted for.

(1) Absolute increased size of the germinative compartment. By definition, under the steady state, the number of cells in the compartments does not change with time, cell flux out of the compartment being equivalent to cell flux in the compartment. The increased size of the germinative compartment thus necessarily implies a disturbance of the steady state conditions. Besides, this imbalance between the proportion of cells that repeat the cell cycle time and those that differentiate must be limited in time as the growth is also limited (Fig. 3). As a consequence when the psoriatic lesion is clinically stable, this kinetic perturbation has ceased and the steady state is restored at the new size level.

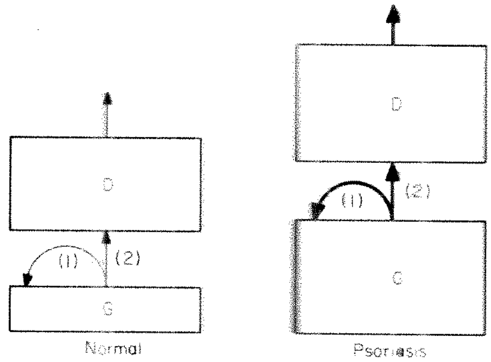

FIGURE 1. Epidermal epithelium contains a germinative compartment (G) and a differentiative compartment (D). In steady state conditions, the fraction of cells which remain in the germinative compartment (arrow 1) is the same as the fraction which leaves the compartment, progressing into differentiation (arrow 2). Psoriasis is characterized histologically by an increased size of the two compartments.

(2) Relative increase in size of the germinative compartment. In psoriasis, the proportion of viable differentiated cells relative to the number of germinative cells is lower than normal. This morphology reflects the fact that (a) the acceleration of cell transit in the differentiated compartment is greater than the acceleration of cell production in the germinative compartment and (b) the differentiated compartment is fed by a germinative compartment that has increased in absolute size.

Biological aspect of our model

It has been demonstrated that keratinization presents two defects in psoriasis (Thaler *et al.*, 1978, 1980; Hunter & Skerrow, 1978, 1981a, b): (1) the persistence into the stratum corneum of two proteins (63 and 55 Kd) which are normally modified in the living cells; (2) the absence of a 70 Kd polypeptide in the stratum corneum and the living cells. Only the first alteration can be induced in an experimental increase of epidermal turnover (stripping).

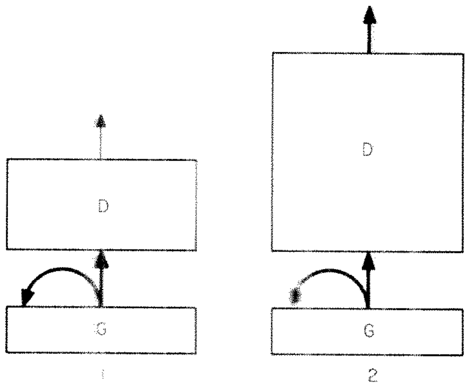

FIGURE 2. A decrease of turnover time of the germinative population without modification of the steady state conditions (pathways 1 and 2 remaining equal) generates growth of the differentiative compartment for a limited period of time. There is no modification of the number of germinative cells.

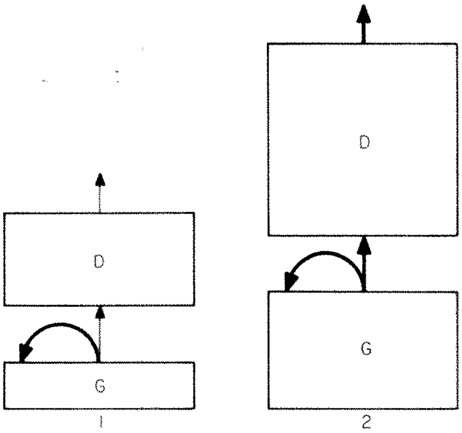

FIGURE 3. The increase in size of the germinative compartment corresponds to an imbalance between the rate of cell production (1) and the rate of cell loss (2) with (1) greater than (2). In the clinically stable lesion, this perturbation has ceased and the steady state is restored at the new level.

This and the observations mentioned above suggested to us the hypothesis of an abnormal keratinization in psoriasis as an independent phenomenon. In this view, the acceleration of transit in the differentiated compartment might simply reflect an abnormal keratinization process that needs less time to be completed than the normal one. Our hypothesis is that psoriasis can be viewed as a differentiation disease with hyperproliferation as a compensatory consequence. This hypothesis is supported by the following observations.

(1) Thinning of the stratum granulosum precedes abnormal proliferation of epidermal cells (Stankler, 1977).

(2) In the initial psoriatic lesion, increased rate of corneocyte formation is the first noticeable event (Johannesson & Hammar, 1981).

(3) In the incipient lesion of psoriasis, the epidermis has no features characteristic of psoriasis and is sometimes thinned (Chowaniec & Jablonska, 1979).

A consequence of our hypothesis is that the skin in psoriatic patients is also abnormal to a certain extent in uninvolved epidermis. There is now some support for this concept. An abnormal labelling index with [^{3}H]thymidine and abnormal mitotic index have been measured in uninvolved epidermis by many authors (Steigleder & Pullman, 1979; Rowe, Dixon & Forsythe, 1978). Also, stimulation of uninvolved psoriatic skin induces changes in epidermal cell kinetics that are similar to those observed in involved skin (Wiley & Weinstein, 1979). This and the extreme sensitivity of uninvolved psoriatic epidermis to mild trauma (Grove, 1980) demonstrate the unstable nature of epidermis in psoriasis. This instability is clinically amplified by the Koebner phenomenon. To explain epidermal instability in psoriasis, we would like to propose the following model: the germinative population in the uninvolved epidermis of psoriasis patients contains a population of stem cells different from but coexistent with the normal basal cells. Our hypothesis is therefore radically different from that of McCullough & Weinstein (1982, unpublished) who also considered the possibility that an abnormal keratinocyte pre-exists in uninvolved skin but who assumed that this related to cell cycle characteristics which, as we have shown, could be an epiphenomenon. Stimulation through various triggering factors would give them a proliferative advantage over the normal population, resulting in their expansion. As those 'abnormal' keratinocytes differ from normality by differentiation characteristics expressed in the type of keratinization they are able to perform, this leads to the appearance of the particular morphology that characterizes psoriatic plaques.

Ultrastructural and cytochemical studies coupled with biochemical investigation of the keratinization process in basal cells of normal and full-fledged psoriatic plaques might allow this hypothesis to be tested by providing us with the appropriate probes for the search of the assumed abnormal keratinocytes in uninvolved skin of psoriatic patients.

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British Journal of Dermatology (1984) **110**, 246–251.

Correspondence

Eczéma craquelé and systemic carcinoma

SIR, I was interested to read Dr Greenwood's report (*British Journal of Dermatology* 1983, **109**, 227) of a case in which widespread eczéma craquelé was the presenting feature of an adenocarcinoma and in his comment that previous reports had noted this finding with lymphoma rather than a solid tumour.

I have seen a similar woman aged 49 who was admitted with severe back pain. This was caused by bony metastases from a spheroidal-celled carcinoma of the breast. She had marked eczéma craquelé of the shoulders and breasts, the lesions in the latter site being striate. The skin condition responded briskly to local aqueous cream and a corticosteroid/urea combination.

Her other symptoms improved for some time following a radiation menopause and chemotherapy, but she died of metastatic disease some 2 years later.

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Staining of baths by short-contact dithranol therapy

SIR, In the last 3 years a number of regimes for short-contact dithranol therapy in the treatment of psoriasis have been reported (Schaefer *et al.*, 1980; Runne & Kunze, 1982). A major potential advantage of such treatment is its suitability for home use, as stressed by Runne and Kunze, who state that previous methods of treatment appear poor by comparison. We write to report problems of severe staining which we feel may limit the acceptability of this treatment.

Patients were treated once daily with concentrations of dithranol increasing from 0·1 to 3%, with 5% salicylic acid in yellow soft paraffin. The dithranol was removed after 1 hour by bathing or showering. Several patients spontaneously reported severe staining of their bath or shower cubicle and questioning of other patients revealed that this was almost universal. The staining has affected both enamel and plastic surfaces and whilst a few patients report that prompt use of a proprietary bath cleanser will remove most of the stain, the majority of patients have been unable to remove the stains and have been left with baths or shower cubicles which are severely discoloured. Use of household bleach and scouring powders has failed to remove the stains and, in the case of plastics, the bleach and powders can themselves cause further damage.

In common with other workers, we have found short-contact dithranol to be an effective treatment for psoriasis. However, unless the problem of staining can be solved, we feel that treatment at home may not be practicable. We have not experienced similar problems with home use of low concentration dithranol creams and have been unable to trace previous reports of this problem with short-contact dithranol regimes. We would welcome advice as to how the staining may be avoided or removed. At present we suggest that patients be warned of the possibility of irreversible staining of their bath prior to the commencement of treatment, as the prescribing doctor could otherwise be held responsible for this 'side effect' of treatment, the only 'cure' for which may be a new bathroom suite.

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Irritancy of anthralin in patients with psoriasis and widespread vitiligo

SIR, In two recent studies of anthralin irritancy in the treatment of psoriasis there has been agreement that type I skin is slightly more sensitive than types II to IV, although less so than would be expected from clinical observation (Kingston & Marks, 1982; Maurice & Greaves, 1983).

I have treated two female patients, aged 45 years and 53 years, who had widespread long-standing vitiligo and chronic plaque psoriasis of more recent onset. Treatment was started with an anthralin cream 0·1% applied daily. After two applications both patients developed a severe inflammatory reaction with a marked worsening of the psoriasis. The patients were also intolerant of weak tar preparations. Systemic treatment with methotrexate and etretinate was necessary to control the psoriasis.

These two cases suggest that although fair-skinned individuals are almost as tolerant of dithranol as dark-skinned patients, the complete absence of pigmentation predisposes to a marked increase in sensitivity to anthralin irritancy. It seems that the ability to produce melanin does protect to some extent against anthralin irritancy and it would be interesting to know whether the investigators concerned have extended their studies to include patients with vitiligo and those with racially black skin.

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Reply

SIR, It is possible that anthralin irritancy is due to free radical formation by auto-oxidation of anthralin (Martinmaa *et al.*, 1978). Electron spin resonance studies have suggested that melanin behaves as a free radical trapping system (Pathak & Stratton, 1968). Thus complete absence of melanin in vitiliginous skin could predispose to anthralin irritation.

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Reply

Sɪʀ, It is not surprising that Dr Roberts has found two patients with vitiligo who have unusual sensitivity to anthralin. We found that patients with skin type I were the most sensitive, but within this group of ten patients there was considerable variation, ranging from extreme sensitivity to more usual tolerance, as indeed can be found in type IV.

The most important experiment to solve the question as to the protectivity of melanin pigmentation would be a within-subject comparison in individuals with vitiligo by measuring the irritancy to anthralin of the uninvolved skin in depigmented and normally pigmented areas.

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Natural killer cells in lymphomatoid granulomatosis

Sɪʀ, Dr Bos and his colleagues (*British Journal of Dermatology* 1983, **109,** 490) make an interesting observation that large numbers of natural killer cells (Leu 7) were present in a possible case of lymphomatoid granulomatosis. In addition T and B lymphocytes and plasma cells were not identified in the cellular infiltrate.

We have also studied a case of lymphomatoid granulomatosis using monoclonal antibodies (Harrington *et al.*, 1983; Wood *et al.*, 1984). The majority of cells were found to be T11-positive (pan T, sheep red cell receptor); most being T4 (helper) with very low numbers of T8 (suppressor-cytoxic) cells. In our case less than 10% of the lymphocytes expressed Leu 7. While NK cells have a weak sheep red cell receptor, the absence of Leu 7 staining coupled with the positive staining for T4 and T8 indicates that the T11 positive cells were T lymphocytes not NK cells. The T4/T8 ratio in the peripheral blood of our patient was 4·4:1 due to low numbers of T8-positive cells. This is in contrast to the T4/T8 ratio (0·17) in the patient described by Dr Bos.

We would agree that NK cells are rarely seen in such high numbers in any primary cutaneous condition. However, NK cells are a normal component of the B cell secondary follicle and can be seen in large numbers in many lymphomas (Banerjee & Thibert, 1983; Mari *et al.*, 1983). The histological criteria to diagnose lymphomatoid granulomatosis must be exact. These include an angiocentric and angiodestructive cellular infiltrate with atypicality (Liebow, Carrington & Friedman, 1972). We note that angiodestructive properties were not reported in the case by Dr Bos.

We feel that the possible differential diagnosis of malignant lymphoma has not been reasonably excluded by the evidence presented. However, we would agree that the observation of high concentrations of NK cells in any lymphoproliferative disorder is of immense importance.

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Cʜʀɪsᴛɪɴᴇ Hᴀʀʀɪɴɢᴛᴏɴ

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Hyperaemic hypoxia in venous ulceration

Sir, In his Comment on venous leg ulcers, Dr Burton (*British Journal of Dermatology* 1983, **109**, 229) has pointed out that the main cause for venous ulcers seems to be a diffusion block for oxygen and other nutrients due to a fibrin barrier around the dilated capillaries, which appear to have proliferated. This concept is supported by our hitherto unpublished findings with transcutaneous oxygen measurements before and after oxygen inhalation, and by investigations of the dermal blood flow by Laser–Doppler.

Measurement of transcutaneous oxygen pressure (tcPO$_2$) (Huch & Huch, 1974; Eberhard *et al.*, 1976) was carried out in the region of leg ulcers, using a skin electrode of the Clark type (Radiometer TCM 1 TC, Copenhagen) operating at a local temperature of 44°C. Nine patients with venous ulcers were compared with nine patients with arterial ulcers. Both groups showed severely decreased basal values of tcPO$_2$ (12·9 ± 12·5 mmHg and 9·7 ± 11·2 mm respectively) compared with normals (59·6 ± 11·2 mmHg).

However, after inhalation of pure oxygen for 3 min, there was a marked increase in tcPO$_2$ in the venous group (to 123·8 ± 97·8 mmHg, $P < 0\cdot01$), but far less in the arterial patients (to 27·0 ± 25·1 mmHg, $P < 0\cdot05$) (Fig. 1). In venous ulcers, in which there is an unimpeded arterial circulation, an increase of the intravascular oxygen tension will readily cause an augmentation of diffusion. In arterial ulcers the restricted increase of tcPO$_2$ after oxygen inhalation can be explained by a deficiency of oxygen transport to the site. These experiments confirm Browse's theory that the local hypoxia in venous ulcers is not caused by a lack of intravascular oxygen transport but by a pathological diffusion barrier around the capillaries

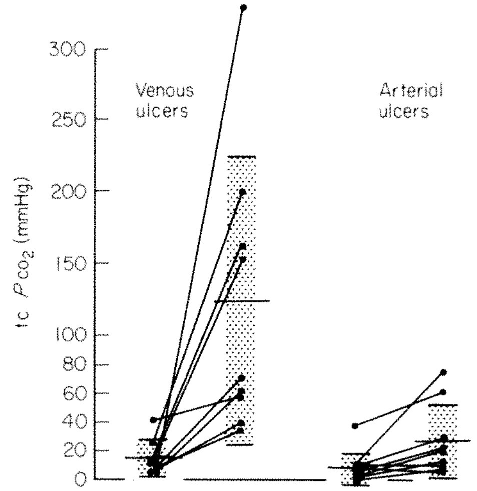

FIGURE 1. Transcutaneous oxygen pressures (tcPO$_2$) before and after inhalation of pure oxygen for 3 min.

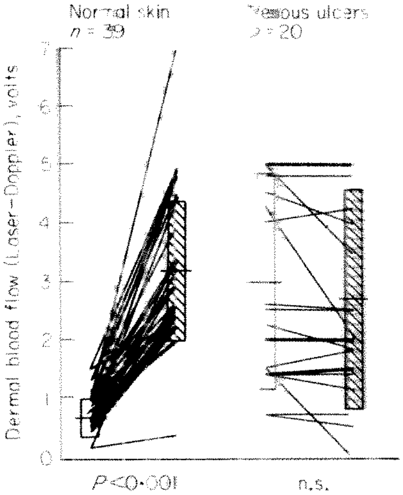

FIGURE 2. Dermal blood flow measured by the Laser–Doppler instrument, before □ and after ▨ local heating to 40° C.

(Browse & Burnand, 1982). These fibrin cuffs around capillaries seem to correspond to the halo formation shown by vital microscopy (Fagrell, 1979)

Some controversy exists regarding the number of dermal capillaries in the ulcer region. Some authors claim they are reduced (Leu, 1965; Fagrell, 1979; Borzykowski & Krahenbuhl, 1981; Franzeck *et al.*, 1982), but other groups report that the local dermal capillary bed is enlarged (Whimster, 1956; Ryan, 1969; Burnand *et al.*, 1981). The probable explanation for this discrepancy is the fact that in histological preparations even a few capillaries can appear proliferated due to their coiled glomerulus-like appearance. However, the size of the capillary bed per unit of dermal tissue volume is enlarged. This capillary dilatation due to an increased venous pressure (ambulatory venous hypertension) is probably the cause of the decreased resistance to flow and consequent hyperaemia which can be detected by several methods (e.g. ^{133}Xe-clearance, intra-arterial injection of labelled microspheres, thermography, photoplethysmography).

The Laser–Doppler instrument (Periflux, Nilsson *et al.*, 1980) is of help in the assessment of dermal microcirculation. The instrument's output is proportional to the velocity and number of blood cells crossing the laser beam in a tissue volume of about 1 cm^3. In normal skin the resting flow is low, but shows a more than threefold increase after local heating to 40° C. In the skin surrounding venous ulcers, the resting flow values are the same as in hyperaemic normal skin and there is no further increase after local heating (Fig. 2). These findings suggest there is maximal vasodilatation in venous ulcers. Thus local hypoxia in venous ulceration is probably not caused by a deficiency of blood flow in the capillaries (Borzykowski & Krahenbuhl, 1981; Franzeck *et al.*, 1982) but is associated with hyperaemia. As Burton says, 'We must now drop the word "stasis" from our ritual incantation for students and replace it with the phrase "pericapillary fibrin deposition".'

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Book Reviews

Practical Dermatology. 4th edn. By I.B. SNEDDON and R.E. CHURCH (1983) London: Arnold. Pp. 244. Price £8.00.

This admirable little book has in many ways been the standard textbook for the undergraduate. Its great virtue has always been that it is about dermatology as it has been seen and practiced by its authors. On the other hand, that very strength is also a potential weakness. Overall, it is an excellent book, but there are many minor points with which one would disagree.

At the very beginning of the book, skin eruptions are classified into four main groups from the treatment aspect, namely those that will recover if nothing is done, those which require inert and soothing preparations, infections and diseases which are uninfluenced by treatment. It seems astonishing that the anti-inflammatory action of corticosteroids and perhaps even the anti-mitotic effect of drugs is left out of this list. It seems a rather charming anachronism that molluscum contagiosum is apparently spread in Turkish baths! In the treatment of 'basal celled epithelioma' (*sic*) 5-fluorouracil is mentioned; but cryotherapy, which is much to be preferred, is not mentioned at all. Under the heading 'In the most severe cases of eczema' the only treatment mentioned is coal tar and zinc ointment BPC. There is a whole long section on how to give Bisgaard therapy—surely doctors are not going to do that themselves. The treatment for very severe scalp psoriasis is apparently Ung. Pyrogall Co. There is quite a lot about polythene occlusive dressings which perhaps would be better left out of such a textbook, though there is a phrase warning about their prolonged usage, and it is good to see that they do warn against the dilution of steroids. Surely prednisolone 5 mg at night is not a good idea for psoriatic arthropathy since it is sufficient to lead to adrenal suppression and to problems with the psoriasis. Finally, it is probably not a good idea to regard anogenital warts as all being venereal.

In conclusion, this is a delightful small textbook, but one would wish to see a number of changes and an expansion of the treatment side since this is what a general practitioner would require.

M.KLABER

Photoimmunology. Edited by J.A.PARRISH, M.L.KRIPKE and W.L.MORISON (1983) New York: Plenum. Pp. 304. Price $39.50.

John Parrish writes in his introduction that photoimmunology is an 'intersection' of photobiology, dermatology and immunology. But to continue the crossroads metaphor it soon becomes clear on reading this book that immunology is a mere footpath to the major road junctions of dermatology and photobiology. What this book should do is to draw attention to the wide scope for work in this field for immunologists.

Chapters 1 to 5 are useful introductory essays on general and dermatological photobiology and can be recommended, e.g. John Spikes on mammalian cell photosensitization. None feature immunology to any extent, despite the chapter headings. Chapter 5 shows action spectra for skin erythema and melanogenesis (p. 81), but the experimental data seem to be lacking references, an unfortunate oversight as the results seem to differ in interesting particulars from previous work, e.g. by Berger *et al.* (1968). (Missing reference is Parrish, J.A. *et al. Photochem. Photobiol.* 1982, **36**, 18[illegible]

It is not until after some ninety pages that a sizeable bolus of immunology arrives—Streilein's and Tigelaar's Chapter 6. Now photobiology is wanting! But theirs is a weighty chapter meriting careful reading and one should not be daunted by new technical jargon and acronyms.

In Chapter 7 experimental photocarcinogenesis is well reviewed by Donald Forbes and Ronald Davies. The immune system in this and related matters is dealt with by Margaret Kripke and colleagues in two interesting and rewarding chapters. Warwick Morison eventually completes the book with five chapters on clinical aspects; here we have much sensible writing and balanced conclusions. He also reviews the somewhat impoverished area of the effects of UVR on human immune reactions.

One was frustrated by citations to unpublished or unrefereed work, viz. 'in press', 'submitted for publication', 'in abstract', 'poster at scientific meeting'. This smacks of ephemeral journalism—to be deprecated in a serious textbook.

The book is a stylish manufactured Plenum Press product with pleasant type face, clear drawings and diagrams; but some chemical equations were a little confusing, viz. equation 8 on p. 246. Some unimportant misprints were mostly in reference lists. The index could not be faulted.

The dust-cover blurb claims the book to be a 'major synthesis' but, excepting photocarcinogenesis, it could be argued that the immunology has yet to start and that 'photoimmunology' is a premature appellation. To be fair, this is a book of good reviews similar to others already available in the last year or two from a number of other publishers, including Plenum Press itself.

I.A.MAGNUS

REFERENCE

BERGER, D., URBACH, F. & DAVIES, R.E. (1968) The action spectrum of erythema induced by ultraviolet radiation. Preliminary report. In: *XIII Congressus Internationalis Dermatologiae* (Ed. by W.Jadassohn and C.G.Schirren) Vol. 2, p. 1112. Springer-Verlag, Berlin.

Proceedings of the Second Fisons Food Allergy Workshop. Chairman, R.R.A. Coombs. (1983) Oxford: The Medicine Publishing Foundation. Pp. 143.

This booklet sponsored by Fisons Pharmaceuticals, who market sodium cromoglycate, is a useful update on the controversial subject of food allergy. It is based on a workshop held in Harrogate in January 1983 under the distinguished chairmanship of Professor R.R.A.Coombs. Five sessions with some forty papers cover the following topics: Advances in pathogenesis and mechanisms; Investigative methods; Clinical conditions—allergic or not?; Management, diet and drugs; Eczema and migraine as a manifestation of food allergic disease.

An interesting paper describes two patients with total allergy syndrome, both of whom had generalized pruritus, transient erythemato-papular skin rashes and C3 deficiency. In another paper, Dr Robert Warin discusses the mode of action of food additives in urticaria, and suggests a pharmacological rather than allergic reaction. Dr D.J.Atherton of Great Ormond Street, in a review of 100 atopic children, presents evidence to show that ten out of twenty-five atopic children who failed to respond to topical therapy were improved on an oligo-allergenic diet for 3 weeks. However, on re-introduction of a normal diet over a period of 6 months, there was no recurrence of the eczema. Dr R.Lever and Professor R.Mackie present evidence that 50% of atopic adults in a small open study trial improved on oral sodium cromoglycate.

The organizers of the workshop found an increasing interest in food allergy in the medical profession, but there is a lot more basic clinical research required to elucidate the food alllergy jigsaw puzzle to convince the sceptic. For those interested in the subject of food allergy, this is essential reading and should be in departmental libraries.

W.N.MORLEY

Quantitative Dermatoglyphics—Classification, Genetics, and Pathology. Oxford Monographs on Medical Genetics No. 10. By D.Z.LOESCH (1983) Oxford: Oxford University Press. Pp. 441. Price £30.00.

Forty years on, this book is a successor to the 1943 classic introduction to dermatoglyphics *Fingerprints, Palms and Soles*, by Cummins and Midlo and it also updates the excellent 1968 work, *The Genetics of*

Dermal Ridges, by Sarah Holt. Basically this book deals with the classification, genetics, and pathological variation of dermatoglyphic patterns. As the author indicates, the first part of the book deals with the theoretical basis of the topological classification initiated by L.S.Penrose and includes the developmental aspects of ridge systems, principles and nomenclature of the method, and its applications to the study of normal variation in human populations. An account of measurements used in dermatoglyphic analysis is also given, including a proposed classification of minutiae for genetic and clinical purposes. In the second part, the principles, methods and results of genetic analyses of dermatoglyphic patterns and measurements are presented. The third part discusses the distortions of ridge patterns in congenital malformations of limbs, in some chromosomal abnormalities, and in some other malformations and diseases. References, which are extensive, are placed at the end of the book just before the index. Comprehensive appendices occupy sixty-nine pages.

The book is a welcome addition to the dermatoglyphic literature and Professor Loesch is to be congratulated on her painstaking work. The book obviously has a limited appeal but it will be of interest to all those working in anthropology and human genetics. Dermatology departmental libraries should obtain a copy. Trainees in dermatology should have a basic knowledge of the genetics of dermal ridges and this book provides this and very much more.

JULIAN VERBOV

Color Atlas and Synopsis of Clinical Dermatology. By T.B.FITZPATRICK, M.K.POLANO and D.SUURMOND (1983) New York: McGraw Hill. Pp 41[illegible]. Price £24.95.

The Atlas is intended as an introduction to Dermatology. Each disease is described under clear headings with the salient features listed. Approximately ninety common skin diseases are covered and illustrated by 148 excellent colour photographs with a brief thumb nail sketch of each patient's history. There are also thirty line drawings. However, the layout is somewhat curious, with 76 blank pages scattered throughout the book. Some of the suggested treatments are controversial e.g., page 55 'Psoriasis improved under corticosteroid creams with plastic occlusion at night'. The last seven pages deal with melanomas and the Harvard recommendations. The book certainly succeeds in its purpose. But why 76 blank pages?

J.E.WHITE

British Journal of Dermatology (1984) **110**, 255–256.

News and Notices

The Bristol-Myers Awards

We are pleased to announce that through the generosity of Bristol-Myers Pharmaceuticals, a sum of money has been made available to the B.A.D. annually for educational purposes, for 3 years in the first instance. This money will be used for the Bristol-Myers Awards. These comprise:

(1) A research travelling scholarship open to Senior Registrars or Consultants to be used by a candidate who is travelling abroad for a specified item of research or training. It is envisaged that the scholarship will be for a period of about 3 months. Value £3,000.

(2) There shall be two travelling fellowships for Consultants. Value £750 each.

(3) There will be three literary prizes which will donate the sum of £400 to be used either for the purchase of books or for subscriptions to dermatological journals. These prizes will be as follows: (a) two awards for the best oral presentations at the Annual Meeting; (b) one award for a highly meritorious paper in the B.J.D. over the previous year submitted either by an individual B.A.D. Member or a unit.

(4) There will be donated a clinical photographic equipments package (two or three per year) to non-teaching units.

Nominations are invited for items 1, 2 and 4 above. Applicants are requested to give sufficient detail to help the Selection Committee to come to a fair decision. Applications should be sent to the Honorary Secretary, B.A.D. Office, 7 John Street, London WC1N 2ES by 20 February 1984.

The Bristol-Myers Literary Prizes for the best oral presentations at the Annual Meeting 1983 were awarded to Dr Stephen Breathnach and Dr John Harper.

The Whimster Prize

Nominations are invited for the above Prize set up to commemorate the late Dr Ian Whimster. It will be awarded on the basis of work (including theses) published in the preceding 3 years in the field of experimental pathology of the skin. Nominations already received will be considered in 1984. Nominations should be sent to the Honorary Secretary, B.A.D. Office, 7 John Street, London WC1N 2ES. The closing date will be 20 February 1984.

The Wycombe Prize 1984

The judges decided that the awarding of this Prize should be deferred for a year and should be awarded for the first time in 1984. Applications are invited for this Prize which is offered for an outstanding contribution to dermatology published from a district general hospital or non-teaching centre in the United Kingdom. Nominations already received will be considered in 1984. For regulations please contact the Honorary Secretary, B.A.D. Office, 7 John Street, London WC1N 2ES. The closing date will be 20 February 1984.

Fellowship of the Royal College of Physicians of Edinburgh

The following Dermatologists have recently been elected F.R.C.P. (Edin.): P.J.C.Ashurst, S.E.Farraj, A.G.Knight, Myra McKelvie, W.D.Paterson, and L.Ranasinghe.

New journal—Pediatric Dermatology

The first issue of *Pediatric Dermatology* appeared in July 1983. It will appear quarterly. The editors are Dr L.M.Solomon and Dr Nancy B.Esterly. The publishers are Blackwell Scientific Publications Inc., 52 Beacon Street, Boston MA 02108, U.S.A.

European Society for Comparative Skin Biology, Grenoble, July 1984

The Fourth International Conference organized by the European Society for Comparative Skin Biology, will take place in Grenoble, France, from 3 to 6 July 1984. Details may be obtained from Professor P.Sengel, Laboratoire de Zoologie et Biologie animale, Université scientifique et médicale de Grenoble, BP53, F-38041 Grenoble, Saint-Martin de Heres, Isère, France.

Skin Pathology Course, Toronto, July 1984

The Dermatopathology Foundation (12th Annual) Course in Gross and Microscopic Pathology of the Skin will be held at the Delta Chelsea Inn, Toronto, Canada, on 9–13 July 1984. For further details please contact the Dermatopathology Foundation, P.O. Box 377, Canton, MA 02021, U.S.A.

17th World Congress of Dermatology, West Berlin, September, 1987

The 17th World Congress of Dermatology will be held in West Berlin on 20–25 September 1987. For further information please contact: Professor C.E.Orfanos, Department of Dermatology, University Medical Centre, Hindenburgdamm 30, 1000 Berlin 45, Federal Republic of Germany. Tel. 030 7982282, 7982808.

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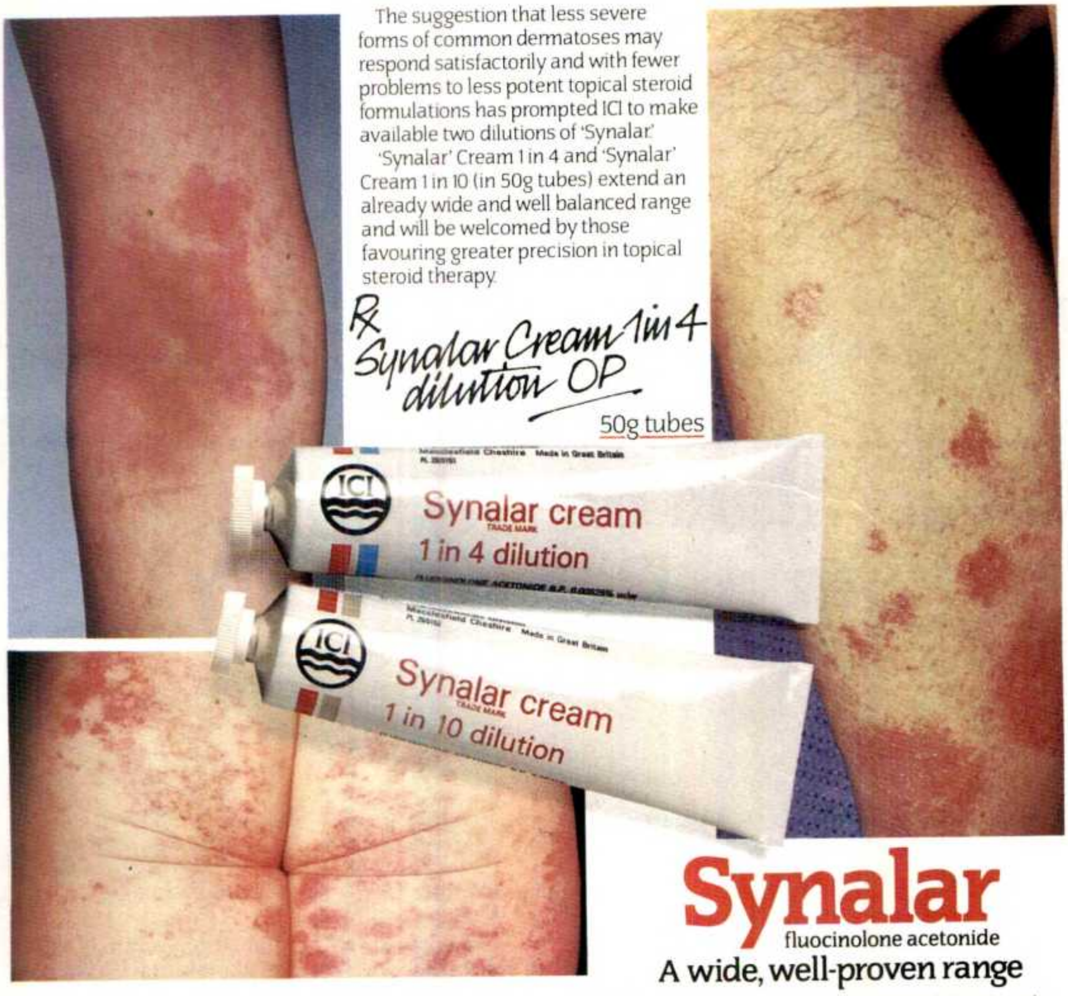
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Textbook of Dermatology

Edited by **Arthur Rook** MD, FRCP *Consultant Dermatologist, Addenbrooke's Hospital, Cambridge*

D.S. Wilkinson MD, FRCP *Consultant Dermatologist, Aylesbury and High Wycombe Hospital Group*

and **F.J.G. Ebling** DSc, PhD *Professor of Zoology, University of Sheffield*

'This latest edition continues its deserved place as a comprehensive encyclopedia of the scope of dermatology with a vast array of disease states presented to the reader. All dermatologists ... and all medical libraries, should reserve a place on their bookshelves for this classic text.' *Journal of the American Academy of Dermatology*

'Anyone with the slightest interest in the skin cannot do without access to it.' *British Medical Journal*

'Rook, Wilkinson and Ebling have compiled an excellent edition of what has rapidly become a classic textbook of clinical dermatology. It is truly the dermatologists' dermatology book.' *New England Journal of Medicine*

Third Edition, 1979. 2448 pages, 1131 illustrations.
Two volumes, £95.00

BLACKWELL SCIENTIFIC PUBLICATIONS LTD
Osney Mead, Oxford OX2 0EL, England

Essentials of Industrial Dermatology

Edited by **W.A.D. Griffiths** MD, MRCP
Consultant Dermatologist, St. John's Hospital for Diseases of the Skin and Senior Lecturer, Institute of Dermatology, London

and **D.S. Wilkinson** FRCP
Consultant Dermatologist, Wycombe General Hospital

This compact handbook provides guidelines and sets out basic principles in the field of industrial dermatology for doctors working as full- or part-time factory doctors, who may have little previous dermatological training. It will also appeal to doctors working in public or community health, particularly in the developing countries where dermatological problems are becoming increasingly apparent. The text includes line drawings and tables for easy reference.

Contents: A national problem *W.A.D. Griffiths;* Industrial dermatitis today and its prevention *D. Burrows;* Constitutional skin diseases in industry *J. Cotterill;* Investigation of industrial dermatitis *C. Hindson;* Soluble oils *R. Rycroft;* Hand eczema in industry and the home *R. Church;* Chemical acne *D. Williamson;* Principles in dermatitis litigation *R. Bursell;* Conclusions and key points *D.S. Wilkinson & W.A.D. Griffiths.*

Spring 1984. 150 pages, 20 illustrations. About £10.00

Blackwell Scientific Publications

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This Journal is covered by *Current Contents, ISI/BIOMED, Science Citation Index* and *ASCA*.

Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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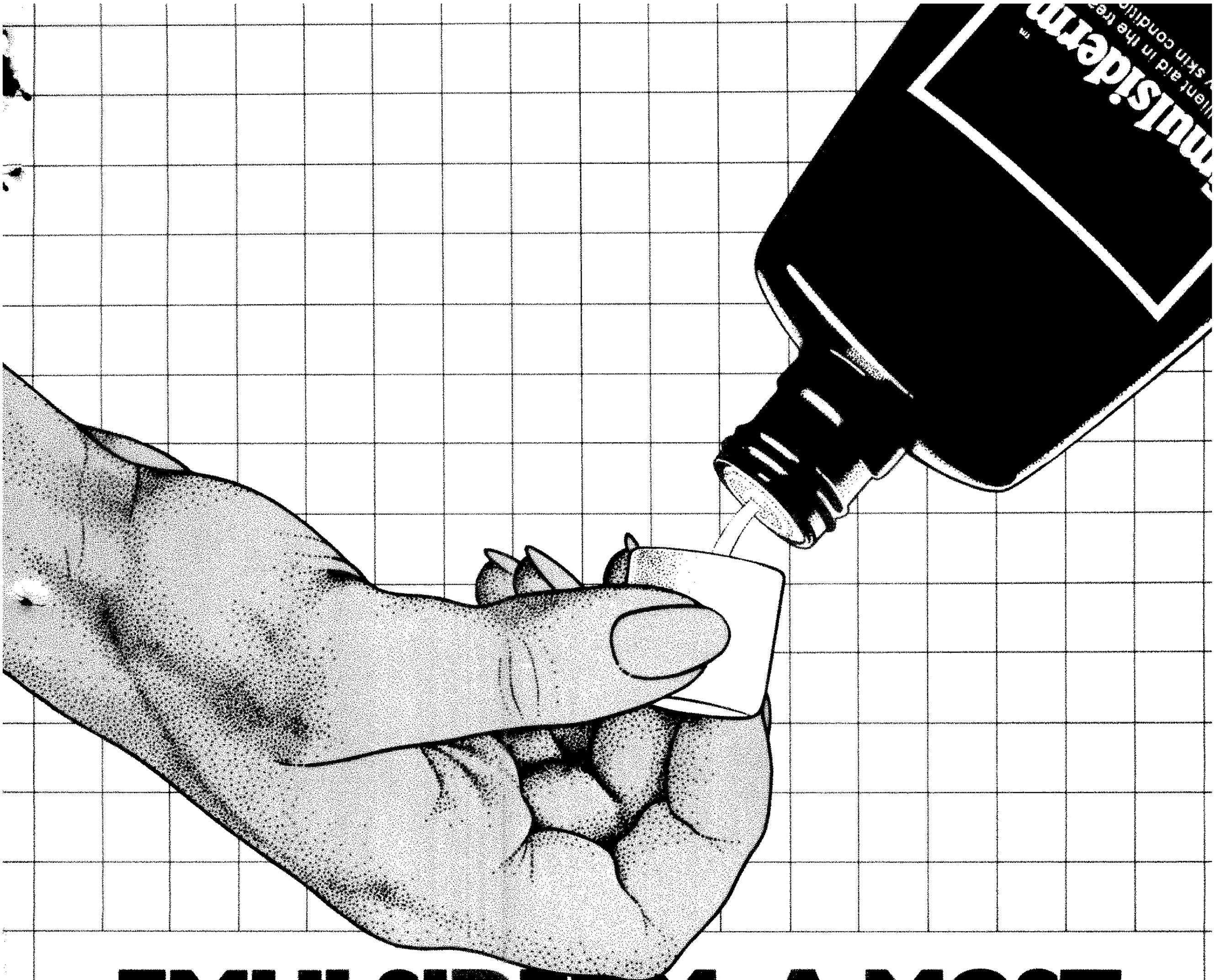

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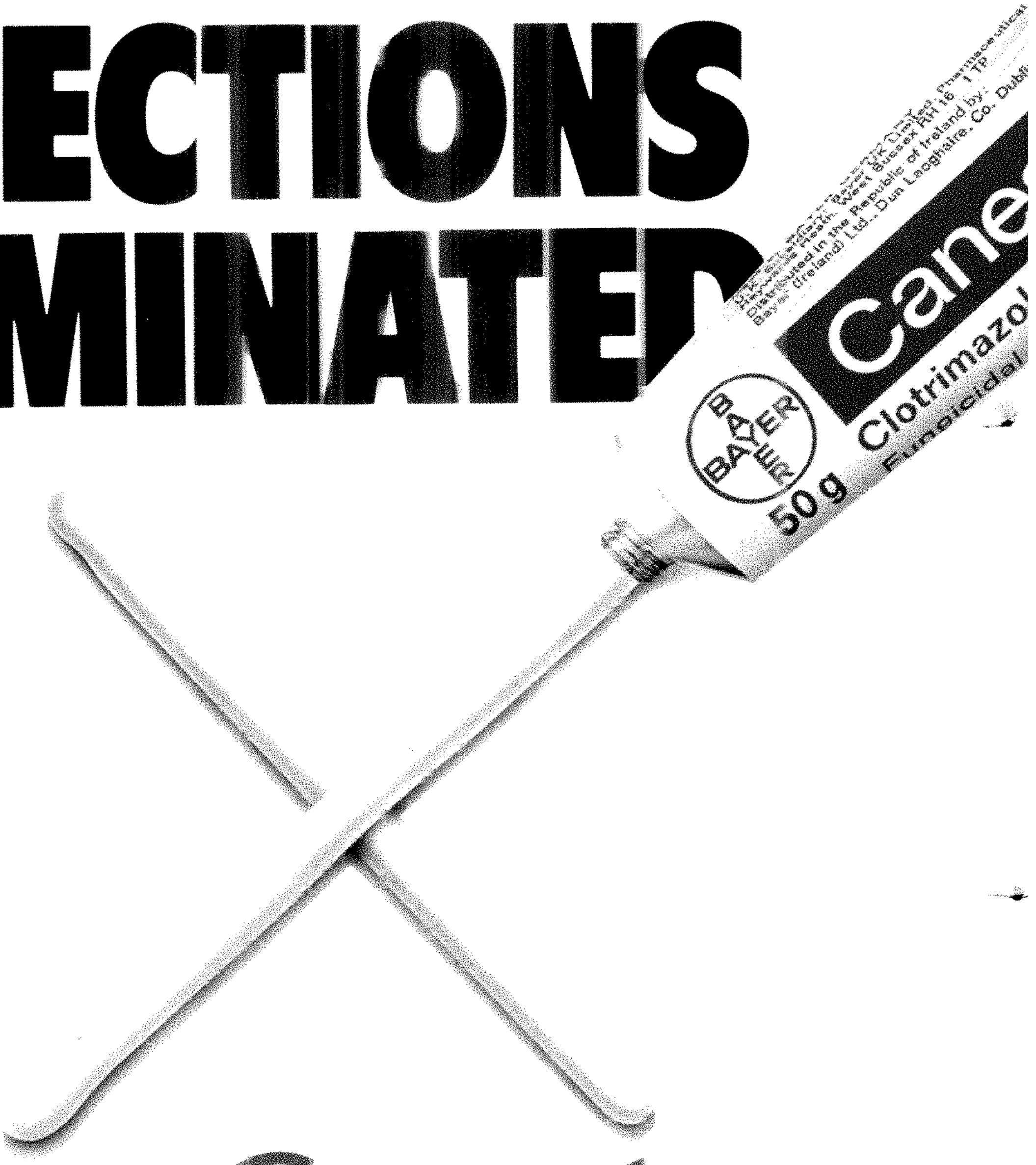
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Side effects

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Contra-indications

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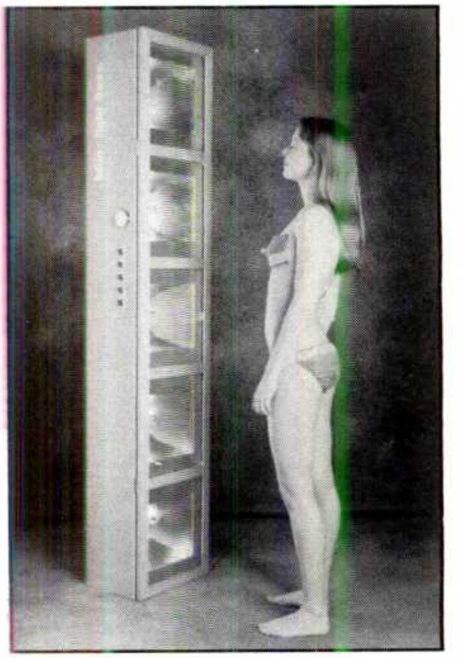

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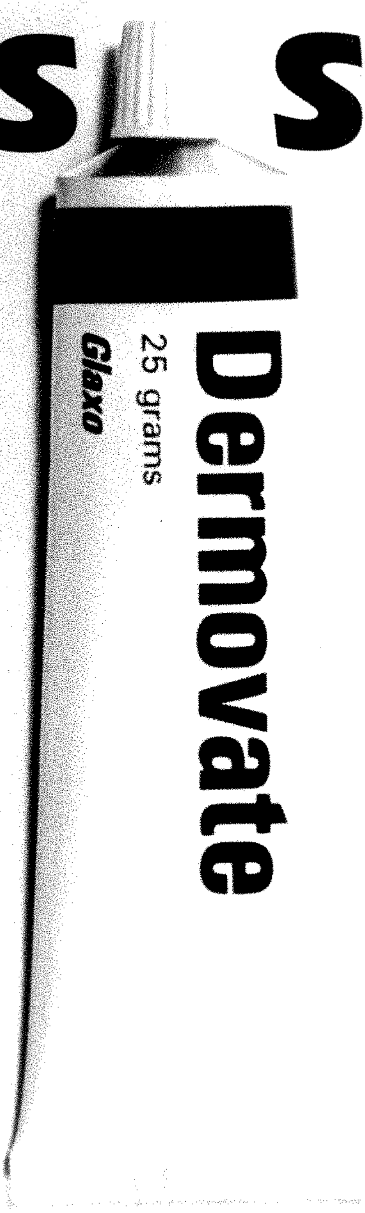

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Edited by **R. Baran** MD, *Head of Dermatological Unit, General Hospital, Cannes,* and **R.P.R. Dawber** MA, MB, ChB, FRCP, *Consultant Dermatologist, Oxford Hospitals and Clinical Lecturer in Dermatology, University of Oxford*

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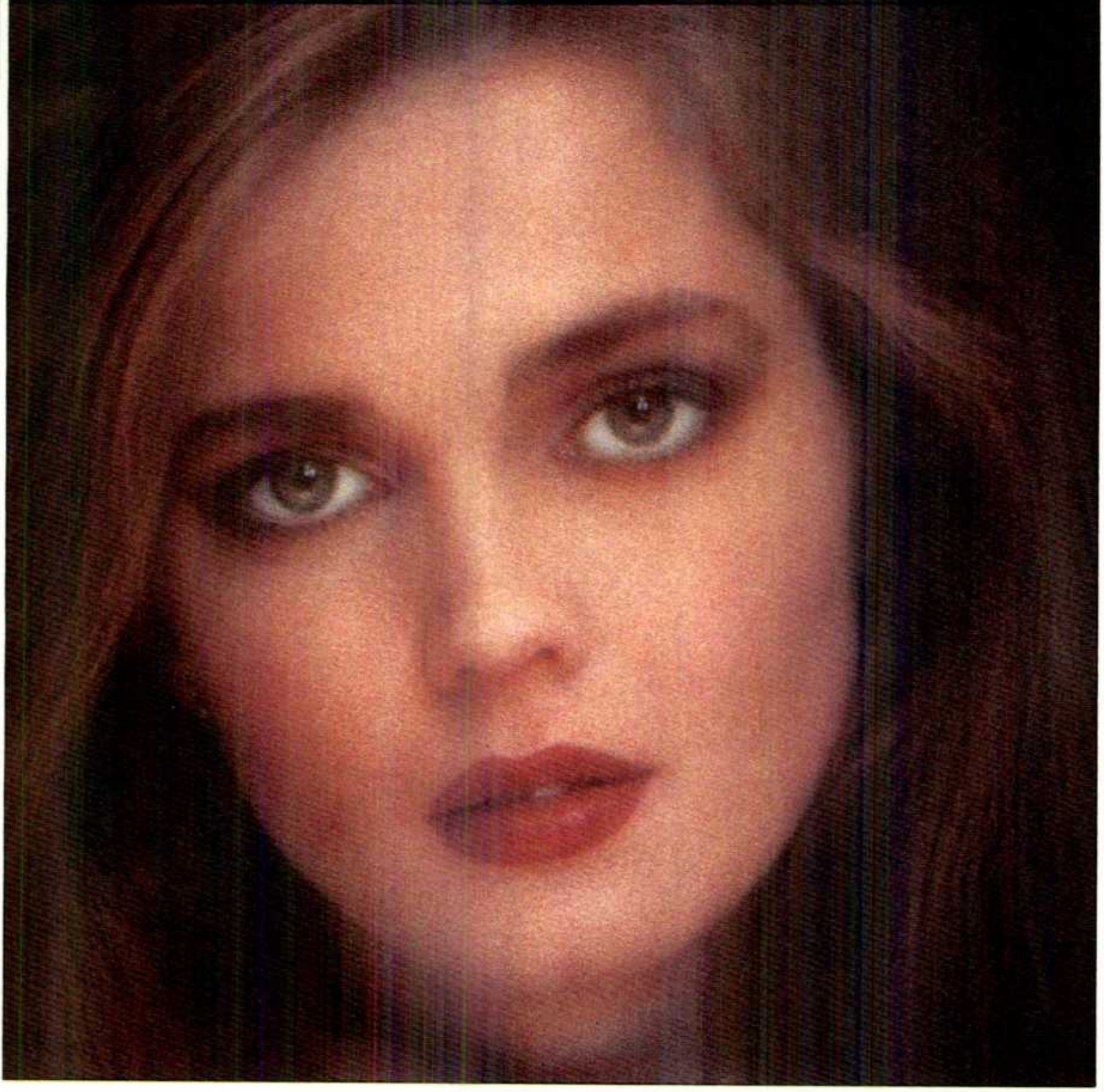

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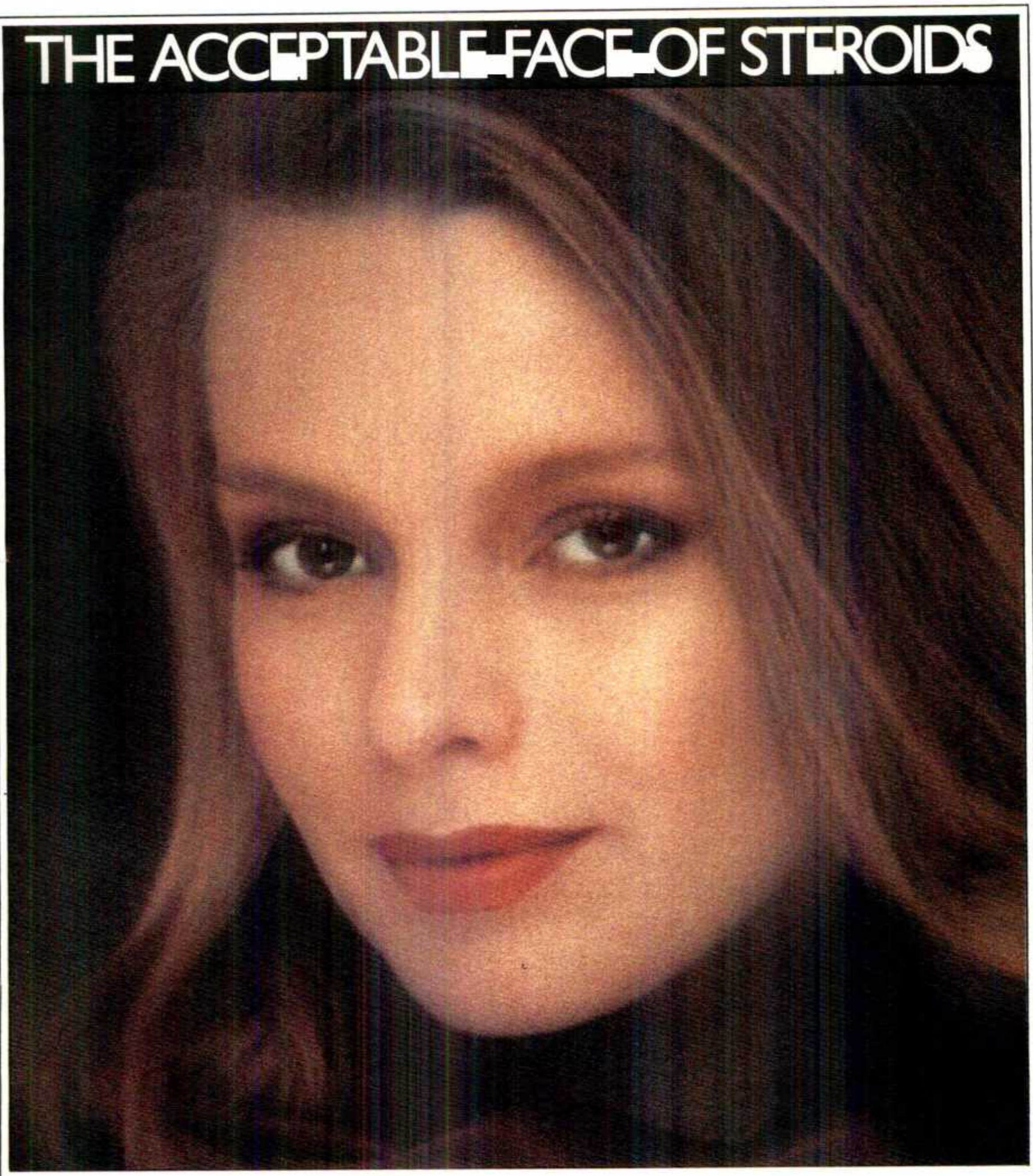

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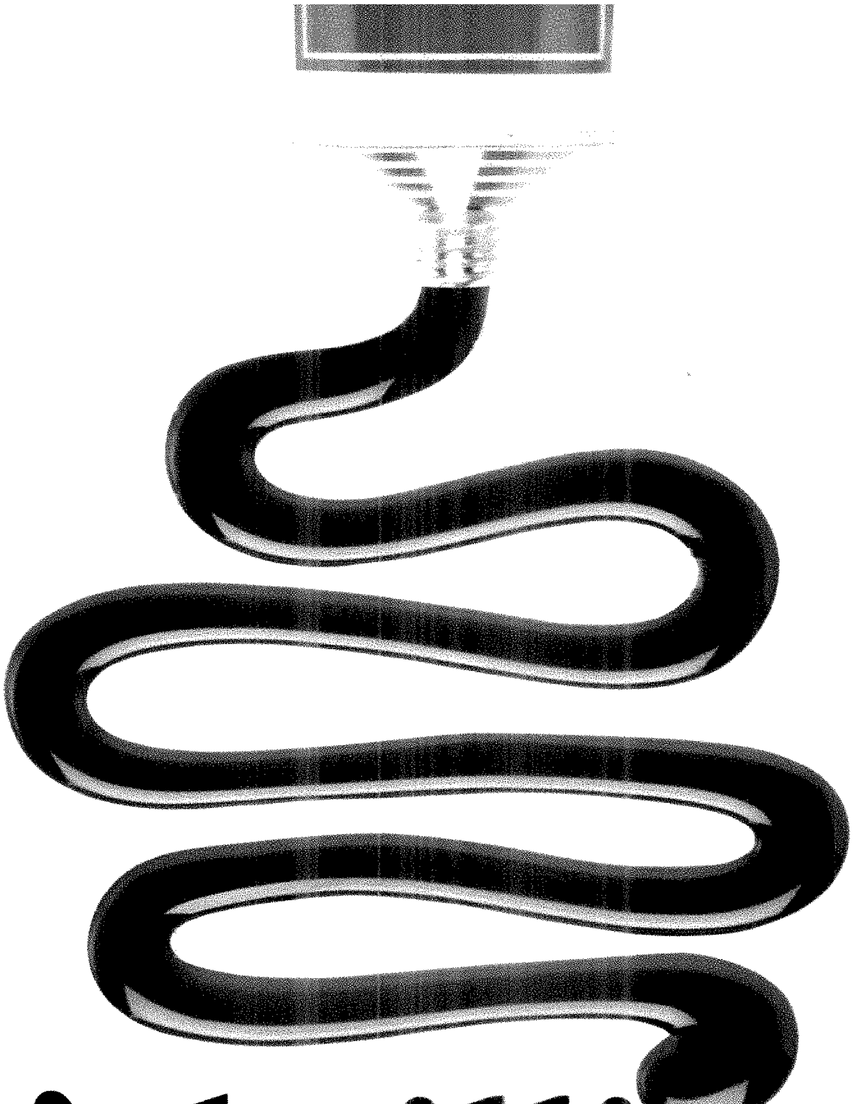

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British Journal of Dermatology (1984) **110**, 257–263.

Clinical and Laboratory Investigations

Dynamics of Langerhans cells in genetically defined murine epidermal cell culture

RHONNA L.COHEN*‡ AND D.A.CHAMBERS†‡

Center for Research in Periodontal Disease and Oral Molecular Biology and Departments of *Oral Pathology, †Biochemistry and ‡Dermatology, Health Sciences Center, University of Illinois, Chicago, U.S.A.

Accepted for publication 10 September 1983

SUMMARY

Unlike keratinocytes, Langerhans cells express both surface ATPase activity and Ia (HLA-DR) antigens. A well-characterized *in vitro* system containing Langerhans cells would be of great use in elucidating their functions. Thus, epidermal cell cultures derived from neonatal Balb/c mice were examined for the presence of Langerhans cells. Twenty-four hours after initiation of culture, ATPase- and Ia-positive cells were seen to be associated with cell aggregates. By day 3, Langerhans cells migrated on to the substratum and, as the cultures matured and stratified, were seen both in groups and as single cells for the duration of the cultures (day 14). During culture, although the total number of cells increased, the percentage of cells expressing Ia antigen and ATPase activity remained constant, suggesting that Langerhans cells increase in number during cell culture. Such a situation could arise from actual division of Langerhans cells during culture or from latent expression of Ia antigen and ATPase activity by pre-existing cells. This is the first study of the dynamics of Langerhans cells in a cell culture system and shows that Langerhans cells are present throughout the lifespan of the cultures.

Recent attention has focused on Langerhans cells (LC) as regulatory cells of bone marrow origin in the skin and oral mucosa (Katz, Tamaki & Sachs, 1979; Frelinger *et al.*, 1979). The presence of Langerhans cell networks within stratified squamous epithelium may provide a means of communication and co-ordination for epidermal cell activities. In addition, although a number of investigators have suggested that Langerhans cells are analogous to skin macrophages (Rowden, Phillips & Delovitch, 1978; Stingl *et al.*, 1978; Shelley & Juhlin, 1976; Hashimoto & Tarnowski, 1968), others have associated Langerhans cells with epidermal proliferative activity (Potten & Allen, 1976). These two functions need not be mutually exclusive. Epidermal cell culture from genetically defined animals provides a model system for the study of Langerhans cell–keratinocyte interaction. This paper describes the dynamics of Langerhans cells in a genetically defined epidermal cell culture system.

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METHODS

Neonatal Balb/c mouse skin was cultured by modification of previously described methods (Marcelo *et al.*, 1978). Briefly, skin was incubated in 0·25% trypsin (Sigma Chemical Co., St Louis, Mo.) in phosphate-buffered saline (PBS), pH 7·3, for 45 min at 37°C. After separation of epidermal and dermal components with forceps, the epidermal cells were released into complete media (RPMI 1640, 13% fetal bovine serum, 10,000 u/ml penicillin, 10,000 mcg/ml streptomycin) and centrifuged through a discontinuous Ficoll gradient for 30 min at 200 × ***g*** at 4°C. Cells that pelleted in the densest fraction were plated in complete medium (1×10^6 cells/ml) on plastic tissue culture dishes (Corning, Cambridge, Mass.) and cultured for periods up to 14 days at 33°C in a humid, 95% air, 5% CO_2 atmosphere. Before assay all cultures were washed in PBS to remove adherent FCS.

For quantitative studies, cells were dispersed by trypsinization (incubation in 0·1% trypsin/0·33% EDTA in PBS for 10 min at 37°C), washed once in PBS and centrifuged for 5 min at 300 × ***g*** at 4°C. Cell viabilities (by Trypan Blue exclusion) were determined and 5×10^5 viable cells were added to microtitre plates for assay. Control studies in which cells were physically dispersed (by a rubber 'policeman') showed cell yields of Ia and ATPase values comparable with those obtained with trypsin/EDTA harvesting.

Ia antigens and ecto-ATPase *in situ* were visualized directly on epidermal cultures grown on glass coverslips. The coverslips were rinsed twice with PBS and treated as described below.

Histochemical staining for membrane ATPase was performed according to the method of Mackenzie & Squier (1975) in which tissue is incubated in a substrate containing ATP, magnesium sulphate and lead nitrate. Controls in which ATP was omitted from the mixture were run in all experiments. In some experiments additional controls omitting lead nitrate were also included.

Detection of Ia antigens performed by indirect immunofluorescence microscopy was performed by a standard method. Monoclonal anti-Ia antibody (kindly provided by Dr G.G.Krueger), was incubated with cultured cells for 30 min at 4°C. After three washes the cells were incubated in the dark with FITC-labelled goat anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.) for an additional 30 min at 4°C. The cells were washed three times and were examined with a Zeiss fluorescence microscope equipped with epi-illumination. Control wells in which the anti-Ia antiserum was omitted were included in all experiments. Antibodies were titrated to give maximum specific binding. All dilutions and washes were made in PBS containing 0·1% sodium azide.

RESULTS

In these studies, murine epidermal cell cultures were examined for the presence of Langerhans cells detectable by ATPase cytochemistry and/or the presence of Ia receptors by fluorescence microscopy. Both of these characteristics have been previously associated in the epidermis specifically with the Langerhans cells. Langerhans cells could be identified throughout the life of the cultures, in the initial plating period and during both the proliferating and differentiating phases of the cultures.

Primary neonatal mouse epidermal cell cultures initially plate as single-cell suspensions, and attach to the culture dish as single cells or as multicellular aggregates within 18 h of plating. Some cells from the aggregates migrate on to the substrate, flatten out and attach. A burst of proliferative activity seen between days 2 and 4 results in outgrowths of polygonal cells which

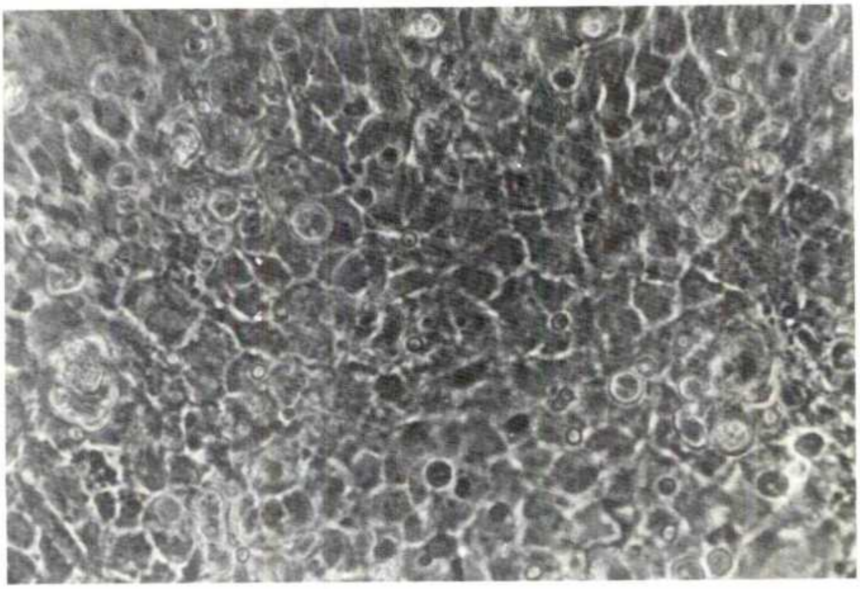

FIGURE 1. Neonatal murine epidermal cell culture, 4 days after culture initiation (phase contrast, × 500).

coalesce into a monolayer. After 4 days the cultures begin to differentiate, as shown by the appearance of tonofilaments and stratification (Fig. 1).

Langerhans cells could be readily demonstrated by both Ia and ATPase markers throughout 14 days of culture. On the first 4 days of culture Langerhans cells were seen to be part of the multicellular keratinocyte aggregates, most showing at least one Langerhans cell (Fig. 2), although positively stained single cells were also seen. After 5–6 days as the cultures became

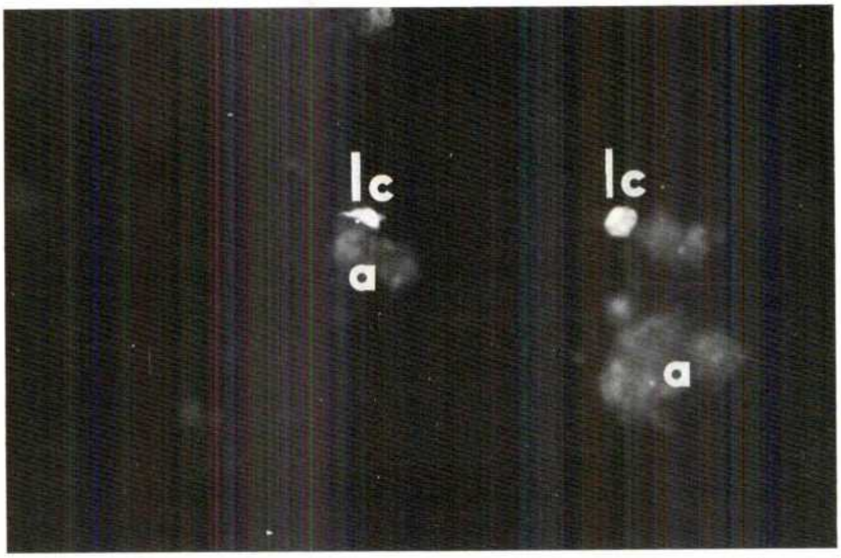

FIGURE 2. Ia-positive Langerhans cells (lc) associated with Ia-negative keratinocyte cell aggregates (a) shown *in situ* in epidermal cell culture 24 h after plating (immunofluorescence, × 320).

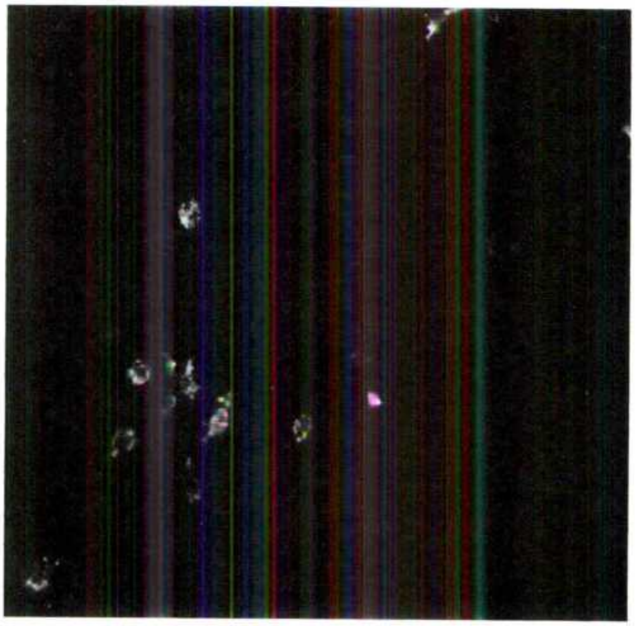

FIGURE 3. Ia-positive cells in 6-day epidermal cell culture. Note the random distribution and variation in outline of Ia-bearing cells. Keratinocytes are Ia negative and cannot be seen in this preparation (immunofluorescence, × 400).

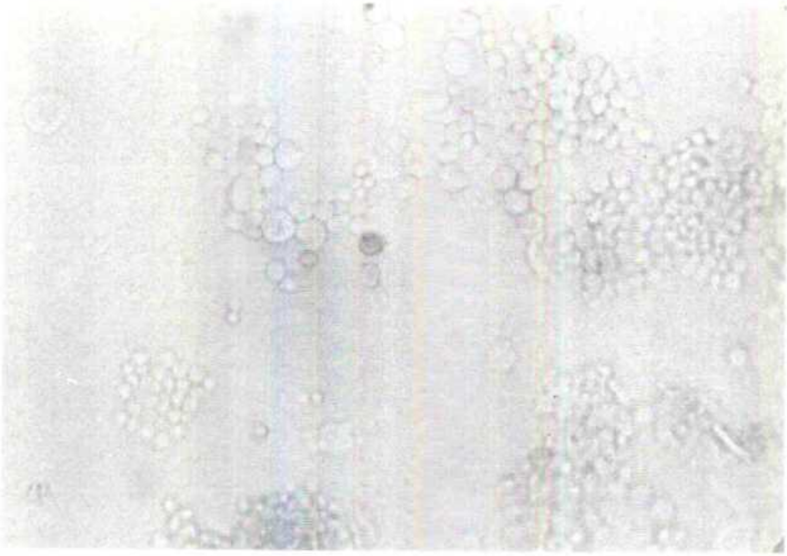

FIGURE 4. ATPase-positive Langerhans cells in cell suspension derived from a 7-day epidermal cell culture (phase contrast, × 250).

TABLE 1. Percentage of positively stained Langerhans cells in epidermal cell cultures measured by Ia and ATPase markers

Days in culture	*n*	% Ia mean (s.e.)	% ATPase mean (s.e.)
1–2	4	2·3 (0·8)	2·8 (0·6)
5–6	3	1·3 (0·1)	1·8 (0·0)
7–8	3	2·0 (0·0)	2·6 (0·0)
9–11	2	1·4 (0·3)	2·4 (0·2)

n = number of experiments; a minimum of 200 cells were counted for each variable in each experiment.

TABLE 2. Comparison between Ia and ATPase positive cells from the same culture

Culture 1 (days)	% Ia$^+$	% ATPase$^+$
1–2	1	2
5–6	1	2
7–8	2	2
Culture 2 (days)		
1–2	5	5
7–8	2	3
9–11	3	3

A minimum of 200 cells were counted for each variable in each experiment.

more differentiated and stratification increased, it was still possible to localize Langerhans cells to an occasional remaining keratinocyte aggregate. More commonly, Langerhans cells appeared to be scattered throughout the cultures. No clear pattern of distribution (Fig. 3) or spatial relationship to keratinocytes was evident. The dendritic morphology characteristic of Langerhans cells *in vivo* was not seen and only occasional cells showed well-developed processes. Most cells were rounded or had short, blunt processes.

In order to determine the number of Langerhans cells present throughout the life of the cultures, it was necessary to use single-cell suspensions for assay (Fig. 4). Table 1 shows that the percentage of ATPase- and Ia-positive cells in cultures up to 10 days remained fairly constant. Using the ATPase technique 2–3% of cells were positive while Ia markers were seen on 1–2% of the cells counted. Thus, good correlation occurs between the two assays, as seen when both methods were used on cells derived from the same culture preparation (Table 2).

DISCUSSION

To our knowledge, this is the first study of the dynamics of Langerhans cells in genetically defined epidermal cultures. In experiments utilizing human epidermal cell cultures, Rubenfeld *et al.* (1981) noted the presence of 3–5% ATPase-positive cells, whereas Morhenn and co-workers (1982) could not identify Langerhans cells after 7 days in culture by ATPase, rosetting procedures or ultrastructural characteristics. Differences reported by a number of laboratories using a variety of species (human, mouse, guinea-pig) may arise from innate species differences or variations in specific culture methods.

In these experiments using a neonatal murine system, Langerhans cells were characterized by their dendritic morphology, the presence of ecto-ATPase and expression of Ia antigen. This report describes ATPase- and Ia-positive cells which were rounded or dendritic in shape, similar to those reported in guinea-pig epidermal cultures (Fritsch, Diem & Honigsman, 1973; Cruikshank & Cooper, 1975). In these murine cultures, the percentages of positive cells obtained by both ATPase histochemistry and Ia antigen reactivity were similar and are within the range reported for mouse skin *in vivo* (Tamaki *et al.*, 1978). The classical criteria generally used for identification of Langerhans cells also include demonstration of intra-cytoplasmic Birbeck granules. We chose not to pursue ultrastructural studies because Birbeck granules in mouse skin are not as numerous as they are in other species (Stingl, 1980) and recent evidence suggests that they may be artefactual in nature (Elofsson *et al.*, 1981). Additionally, Birbeck granules have not been observed in epidermal cultures derived from mouse (N.E.Fusenig, personal communication) or human skin (Morhenn *et al.*, 1982) although they have been reported in guinea-pig cultures (Fritsch *et al.*, 1973).

During culture, although the total number of cells increases, the percentage of cells expressing Ia antigen and ATPase activity remains constant, suggesting that Langerhans cells increase in number. This situation could arise from actual division of Langerhans cells or from latent expression of Ia antigen and ATPase activity by pre-existing cells.

The detailed mechanisms by which Langerhans cells participate in epidermal homeostasis are still obscure. Investigators have associated Langerhans cells with both immunological functions and epidermal proliferation but the evidence for these activities is largely circumstantial. This culture system is ideally suited for studying the effects of pharmacological agents on Langerhans cell–keratinocyte interactions. Preliminary experiments in this laboratory reveal that Langerhans cells are maintained in cultures treated with minoxidil, phenytoin and the phorbol ester 12-*o*-tetradecanoyl phorbol 13-acetate (TPA), drugs known to affect epidermal proliferation (R.L.Cohen & D.A.Chambers, unpublished observations)

The enhanced knowledge of how Langerhans cells participate in skin graft rejection could open new horizons for the use of cultured human skin for clinical transplantation. Some of these avenues are being explored by Krueger and his colleagues using a nude mouse xenograft model (Krueger, Daynes & Eman, 1983) and Elmets *et al.* (1982) employing organ culture. This *in vitro* murine cell culture system in which Langerhans cells can be studied under controlled conditions can complement the work of others using *in vivo* systems, and may help resolve much of the current obscurity surrounding Langerhans cell physiology and Langerhans cell–keratinocyte interaction.

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An improved method for the establishment of cell lines from primary skin melanomas

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SUMMARY

Fourteen explants of primary skin melanomas and six explants of lymph node metastases were used to establish melanocyte cell lines, and in all twenty experiments the melanocytes became overgrown by fibroblasts. By contrast, eight melanocyte cell lines without fibroblast contamination were established by mechanical cell separation followed by either direct plating in microwells (five out of thirteen primary tumours and three out of four lymph node metastases) or plating of cells from colonies grown in soft agar (three out of five cases). It was also observed that cell crowding and partial (as opposed to total) replacement of the culture medium during the early steps of culture favoured the growth of tumour cells. This observation suggests that melanoma cells are subjected to an autocrine type of growth regulation.

Establishment of skin cell lines is frequently accomplished by either explant outgrowths or plating of dispersed, mechanically or enzymatically separated cells (Prunieras, 1975). Two major technical problems frequently encountered in either technique are (1) maintenance of active multiplication of the initial attached tumour cells (primary cells), (2) prevention of overgrowth of normal fibroblasts (Okabe *et al.*, 1979).

This study records the results obtained mainly with primary but also with metastatic malignant melanomas using explant techniques, and both transfer of tumour colonies from semi-solid agar and growth of mechanically separated cells in microtest culture wells in the establishment of cell lines. The importance of the initial cell number to surface area (cell crowding), the amount of spent medium removed and the elimination of fibroblasts using the latter two methods are discussed.

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METHODS

Culturing techniques

A series of malignant melanomas, the clinical features of which are shown in Table 1, were

TABLE 1

	Patient	Site	Sex	Histology	Level (Clark)	Established lines
Explants						
1	M.P.	Thigh	M	NM	V	
2	B.F.	Parotid	M	Met	—	
3	R.L.	Lymph node	M	Met	—	
4	J.S.	Shoulder	M	SS	IV	
5	L.G.	Face	F	HF	—	
6	A.M.	Arm	F	SS	IV	
7	H.L.	Arm	M	SS	I	
8	M.D.	Arm	M	N	IV	
9	D.R.	Back	M	N	IV	
10	C.I.	Back	F	SS	IV	
11	P.J.	Leg	F	NM	IV	
12	M.D.	Back	M	NM	IV	
13	A.B.	Lymph node	F	Met	—	
14	H.L.	Lymph node	M	Met	—	
15	R.A.	Lymph node	M	Met	—	
16	A.M.B.	Lymph node	F	Met	—	
17	E.D.	Arm	F	NM	III	
18	B.R.	Arm	M	SS	III	
19	A.F.	Back	M	SS	IV	
20	R.C.	Back	M	NM	IV	
Mechanical separation						
1	B.F.	Parotid	M	Met	—	
2	S.S.	Arm	F	SS	III	
3	C.N.	Lymph node	M	Met	—	+
4	A.D.R.	Lymph node	M	Met	—	+
5	H.B.	Lymph node	M	Met	—	+
6	R.C.	Back	M	NM	IV	
7	A.F.	Back	M	SS	IV	+
8	B.R.	Arm	M	SS	III	
9	M.D.	Back	M	NM	IV	+
10	R.H.	Leg	M	SS	III	
11	E.D.	Leg	M	SS	III	+
12	D.R.	Back	M	N	IV	
13	F.P.	Arm	F	SS	III	
14	P.J.	Neck	F	NM	IV	+
15	E.L.	Leg	F	SS	III	
16	J.S.	Back	M	NM	V	+
17	E.D.	Leg	F	SS	III	

NM = Nodular melanoma.
SS = Superficial spreading melanoma.
HF = Hutchinson's freckle.
Met = Metastases.

removed at operation and taken immediately to the laboratory where they were washed with PBS. The three culturing techniques used were as follows:

(1) Tumour fragments 1 mm^2 were plated in Corning 25 cm^2 culture flasks using minimal essential medium (MEM) supplemented with 15% fetal calf serum and antibiotics (penicillin 100 i.u./ml and streptomycin 100 µg/ml). Two ml of medium were added on day 1 and 5 ml on day 3. The cells were then fed twice weekly, and one-half of the spent medium was removed and replaced each time.

(2) Dispersed cells were obtained by grating tumour fragments against a 0·5 × 0·5 mm metallic mesh immersed in PBS. Cells were collected and allowed to settle for 15 minutes. The supernatant was aspirated and plated in LIMBRO FB-16-24 TC microtest culture plates containing varying concentrations of cells (100–2000 cells) per well. The wells were then filled to three-quarters capacity or approximately 0·2 ml. During the initial first 3–4 weeks of growth, only one-fourth to one-eighth of the spent medium was removed and replaced twice weekly. After sufficient growth had occurred, half of the spent medium was replaced with fresh medium twice weekly.

(3) Dispersed cells obtained in the same manner as described above were plated in semi-solid agar as described by Courtenay & Mills (1979), except that irradiated cells were omitted. After sufficient growth had occurred (4–5 weeks), colonies of tumour cells were aspirated under sterile conditions and then vigorously pipetted into microtitre wells to disperse the surrounding agar.

Fibronectin and collagen-coated plates were prepared as described by Kramer, Gonzalez & Nicolson (1980) and Kleinman, Klebe & Martin (1981).

Cells were grown in humidified incubators at 37°C with 5% CO_2. Confluent cultures were trypsinized using trypsin–EDTA (0·05–0·02%) and replated in wells having gradually increasing diameters.

Cell identification

Cells were identified by the following techniques.

Inverted Zeiss light microscopy. Malignant melanocytes are typically triangular, dendritic or cuboidal in shape and frequently form 'grape-like' clusters of tumour cells in confluent cultures.

Electron microscopy (Philips 300). The technique for fixation of cells has been described previously (Rougé & Aubert, 1979).

Dopa stain for identifying melanocytes (personal communication with Prof. G.Szabo). Cells fixed for 1 hour at 37° in 5% formol were washed with PBS. Dopa 1 : 1000 (pH 6·8) was added and the cells were incubated at 37° for 1 hour. The solution was removed and a fresh Dopa solution was added. The cells were kept at 4°C for 15 hours prior to reading. Dopa-positive cells were brownish-black in colour.

Leucine aminopeptidase (LAP) for identification of fibroblasts (Nachlas, Crawford & Seligman, 1957). Cells were rinsed three times with Earle's solution and then air-dried for 30 minutes. Sorensen's solution 0·1 mol/l (pH 6·5), NaCl 0·15 mol/l, KCN 0·2 mol/l and fast blue salt 10 mg with and without L-leucyl-β napthylamide 2·73 × 10^{-3} mol/l was added to test cells and controls, then incubated at 37°C for 1 hour. Cells were rinsed and $CuSO_4$–$5H_2O$ (0·2 mol/l) was added for 2 minutes. The reaction was then visualized. Violaceous granules were present in the cytoplasm of LAP-positive cells.

RESULTS

Tumour cells arising from explants were initially seen more rapidly and in greater numbers in cultures from lymph node metastases than in those from nodular and planar melanomas. This was perhaps related to the intrinsic growth rate, absolute cell number per fragment or the stromal structure. Epithelial outgrowths, in cases of cutaneous tumours, were seen at day 4–5, fibroblasts appearing approximately at day 5–10. Tumour cells were consistently overgrown by fibroblasts in all cases as shown by LAP (staining fibroblasts), Dopa (staining melanocytes) and electron microscopy (absence of premelanosomes and melanosomes) on sacrificed flasks.

TABLE 2.

	No. of cases	Primary lines	Established cell lines
Primitive melanomas			
Explants	14	Overgrowth by fibroblasts	—
Mechanical separation, plated in microwells	13	8	5
Lymph node metastases			
Explants	6	Overgrowth by fibroblasts	—
Mechanical separation, plated in microwells	4	4	3
Soft agar			
Plated from colonies, grown in soft agar	5	3	3

As shown in Table 2, mechanical separation and transfer of colonies from semi-solid agar into microtest wells were the most successful methods for obtaining primary and established cell lines. Direct plating after mechanical separation resulted in fibroblast overgrowth in some wells. However, numerous wells were usually found to be fibroblast-free. Prior growth of the tumour cells in agar eliminated fibroblast proliferation.

In several instances cells did not attach to the plastic wells following transfer from semi-solid agar. Use of collagen–fibronectin coated plates did not facilitate cell adhesion.

Electron microscopy of established and primary cell lines demonstrated the presence of both premelanosomes and melanosomes within the cell cytoplasm. Light microscopy demonstrated either dendritic, cuboidal or triangular cells with varying degrees of pigmentation.

The amount of medium and size of the culture flask in relation to the number of primary attached cells was important with respect to cellular growth. In a series of experiments, twenty to forty primary tumour cells were plated in microtest wells and 25-cm^2 flasks with 200 μl and 2 ml of media added respectively. The cells in the larger wells remained in a quiescent state for many weeks with eventual cellular death, whereas confluent growth occurred normally in the smaller wells. In large and small wells with a limited number of primary cells, a complete medium change four times each week usually resulted in cell death. By contrast, partial medium changes favourably influenced cell growth in the microtest wells.

DISCUSSION

With respect to development of tumour cell lines, little has been mentioned in the literature pertaining to the importance of the initial size of the culture wells, frequency and amount of medium changed, or methods in obtaining cells. In our study, the use of microtest culture wells and disassociated cells, obtained directly from tumours or secondarily transferred from colonies grown in semi-solid agar, demonstrated superior results to those obtained using explant techniques in the development of primary and established cell lines. In particular, these methods were successful in developing lines from primary melanomas which are reportedly more difficult to cultivate than cells obtained from metastatic deposits (Aubert *et al.*, 1977).

The initial maintenance of active proliferation of malignant melanocytes was improved by the use of partial medium changes and increasing cell density using microtest wells. Our results obtained by plating a limited number of cells, suggest that tumour cells may influence their own growth by secreting endogenous growth factors into the media. Dilution or frequent removal of these factors inhibits cell growth. One of these peptides produced by melanoma cells, 'transforming growth factor' described by Todaro, is capable of stimulating cell growth, partially replacing the requirement for high cell density and also inducing phenotypic changes in normal cells characteristic of malignant cells (Sherwin *et al.*, 1983; Epstein & Kaplan, 1979; Todaro *et al.*, 1980; Heard *et al.*, 1983). Various other endogenous growth factors and enzymes (i.e., nerve growth factor, sarcoma growth factor, plasminogen activators, proteases, etc.) produced by tumour cell lines have been demonstrated *in vitro* (Wilson *et al.*, 1980). Comparison of low density primary and established lines *in vitro* further demonstrates the importance of the initial cell density. In contrast to primary cells, established lines (50–70 subcultures) will grow in dilute inocula (Paul, 1970). This may be partially explained by the findings in some tumour cell systems which suggest that less than 5% of the original plated cells give rise to cell lines (Gusterson *et al.*, 1981).

Elimination of fibroblast overgrowth is another significant advantage of these techniques. Various methods exist to eliminate contaminating fibroblasts, viz. anti-fibroblast monoclonal antibodies, differential EDTA separation, growth at low temperature, isolation of colonies in microcylinders, etc. (Gusterson *et al.*, 1981; Jensen & Therkelson, 1981; Giovanella *et al.*, 1976). The technique described (plating mechanically separated cells directly into the microtest wells) normally allowed a greater initial number of attached tumour cells within a small surface area, facilitating selective overgrowth by the cells. Sufficient growth of certain neoplastic cell lines may in fact inhibit fibroblast growth by production of factors detrimental to their growth (Weiss & Njeuna, 1971).

Transfer of tumour colonies grown in semi-solid agar eliminated fibroblasts in another fashion. These cells are selectively inhibited from growing in this medium due to their requirement of 'anchorage-dependent growth' (Silinkas *et al.*, 1981). One problem encountered, however, with this technique was related to attachment of the transferred tumour colonies. In several instances the cells resisted attachment to standard culture dishes including collagen–fibronectin coated wells. This was attributed to either selective cloning of cells lacking factors for attachment or a physical barrier of agar still present around the cells.

Finally, with respect to cellular morphology, several reports have described an *in vitro* phenotypic modulation occurring with cells obtained from melanomas, particularly primary tumours (Aubert, Rougé & Galindo, 1980; Aubert *et al.*, 1977). The authors, using explant techniques, described a phase of de-differentiation of tumour cells during the first few weeks of culture into 'fibroblast-like cells'. A spontaneous re-differentiation to cells morphologically identical to the original tumour cells occurred several months later in 25% of cases. In our series, including both primary and metastatic tumour cells, this phenomenon was not observed. The tumour cells constantly retained their original characteristics (dendritic, cuboidal or triangular) throughout the subcultures (Liao, Dent & McCulloch, 1975). Cells piling up into 'grape-like' clusters, typical of transformed cells, were seen in all lines, particularly in confluent cultures.

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Supernatants from cultured human epidermal keratinocytes stimulate angiogenesis

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SUMMARY

Conditioned media from three different strains of human epidermal keratinocytes in culture were assayed on the chorioallantoic membrane of the chick embryo.

Vascular responses were examined 4 days later stereo-microscopically and compared with controls—unconditioned medium and medium incubated with 3T3 cells. Specimens were also collected after 1, 2, 3 and 4 days for serial histological examination. Media from all three strains of keratinocytes stimulated statistically significant vascular growth relative to controls. Leukocytic infiltration was not demonstrated histologically at any time. In addition, quantitation of ectodermal epithelial hyperplasia in experimental groups did not reveal any significant difference when compared with controls. These results have demonstrated that epidermis may directly encourage vascular growth in the absence of significant inflammation.

The epidermis is an avascular, cellular structure separated from its blood supply by an intact basement membrane (Lever & Schaumburg-Lever, 1975); it is also characterized by a relatively high rate of turnover (Aherne, Camplejohn & Wright, 1977). For these reasons it is likely that the epidermis would directly influence dermal vascularity. The association of vessel growth with epidermal proliferation (Ryan, 1970), e.g. in psoriasis, as well as the vascularization of skin grafts (Smahel, 1977) suggest that epidermis may specifically stimulate angiogenesis.

In studies using the hamster cheek pouch bioassay, both epidermal implants separated from the vascular bed by microporous filters and epidermal cytoplasmic extracts elicited vasoproliferation (Wolf & Harrison, 1973). However, in every instance, vessel growth was antedated by substantial leukocytic infiltration, a finding well established as a cause of angiogenesis (Fromer & Klintworth, 1975a, 1975b, 1976; Polverini *et al.*, 1977; Nishioka & Katayma, 1978; Plishkin, Ginsberg & Carp, 1980). Thus, while an epidermal vasoformative factor still might be involved, the results in these studies were confounded by the development of inflammation prior to the onset of vascular growth.

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Since media incubated with a number of tissues and cell types in culture—tumour and transformed cells (Klagsbrun, Knighton & Folkman, 1976), retina (Glaser *et al.*, 1980), macrophages (Polverini *et al.*, 1977), and lymphocytes (Nishioka & Katayma, 1978; Plishkin *et al.*, 1980)—have been shown to stimulate microvascular proliferation when assayed *in vivo*, we postulated that conditioned media from cultured epidermal keratinocytes might produce similar results. In addition, characteristics of epidermal keratinocytes in culture suggested the likelihood of the elaboration of an angiogenic activity by these cells. Cultured keratinocytes are very motile (Sun & Green, 1976; Green & Thomas, 1978) and synthesize large amounts of hyaluronic acid (King, 1981; Brown & Parkinson, unpublished observations) and in these respects resemble the keratinocytes of wounded epidermis *in vivo*. Cultured human keratinocytes also resemble psoriatic epidermis in that they shed nucleated squames into the growth medium (Green, 1977) and produce an incompletely differentiated epithelium (Rheinwald, 1980). It is well established that these circumstances *in vivo*, i.e., wound healing and psoriasis, are associated with new vessel growth (Ryan, 1970). Thus it is possible that analogous epithelial behaviour in culture may well be accompanied by production of a vasoproliferative factor. We report here the demonstration of a non-dialysable, heat-labile activity from media cultured with human epidermal keratinocytes capable of promoting new blood vessel growth on the chorioallantoic membrane of the chick embryo (CAM) in the absence of significant inflammation.

METHODS

Cell culture technique

Human epidermal keratinocytes were cultured from the sources listed in Table 1 according to the methods described by Green and co-workers (Green, 1978; Rheinwald, 1980; Rheinwald & Green, 1975, 1977). Briefly, epidermal cells were grown by co-culturing them with lethally-irradiated Swiss 3T3 cells (Flow Laboratories) in Dulbecco's Modified Eagle's Medium (DMEM) containing 0·4 μg/ml hydrocortisone (Rheinwald & Green, 1975), 100 ng/ml cholera toxin (Rheinwald & Green, 1977) (Sigma Chemical Co., St Louis, Mo., U.S.A.) and 20% (v/v) fetal bovine serum (Sera Lab., Crawley Down, Sussex, U.K.). Epidermal growth factor (Collaborative Research, Waltham, Mass., U.S.A.) was added at the first and subsequent medium changes to give a final concentration of 10 ng/ml (Green, 1978). Second or third cultures were initiated by inoculating 2×10^5 keratinocytes into 9-cm plastic culture dishes (Nunc, Roskilde, Denmark) together with $1{\cdot}2\times10^6$ lethally irradiated 3T3 cells. After 10 days the 3T3 feeders and small numbers of human fibroblasts were selectively removed by exposing the cultures to 0·02% EDTA and then pipetting vigorously (Rheinwald & Green, 1975), a procedure leaving the culture less than 0·1% contaminated with non-epidermal cell types.

TABLE 1. Sources of cultured human epidermal keratinocytes

Strain	Sex	Site of origin	Age of donor (years)
R	Male	Foreskin	10
T	Male	Foreskin	5
W	Female	Abdominal skin	2

Preparation of conditioned media

Cultures together with control dishes containing irradiated 3T3 cells were washed three times with calcium- and magnesium-free phosphate-buffered saline. The epidermal and control cultures were then incubated at 37°C for 4 h with serum-free DMEM (2 ml per dish) in an atmosphere of 5% CO_2 and 95% air. Conditioned medium concentration was expressed as number of cells/ml of DMEM incubated. Samples were then immediately frozen and stored at −20°C until they were used (usually within 2 weeks). Media were then dialysed for 48 h against 0·9% saline with two changes at 4°C and then filtered (Millipore; 0·22 micron pore size).

Chorioallantoic membrane assay

Conditioned media were assayed on the CAM on the 10th day of incubation after preparation of embryonated eggs according to the method of Zwilling (1959), slightly modified. Briefly the developing CAM was allowed to drop away from the eggshell by aspiration of albumin from the egg. A large window in the shell was subsequently created permitting access to the membrane. Two mm Millipore filter discs (type HA, 0·45 micron pore size) saturated with conditioned media were applied to the CAM. Controls consisted of DMEM and Swiss 3T3-conditioned medium prepared in the same manner. A sample of medium from the T strain was heated to 60°C for 30 min as well. Vascular responses were quantitated *in ovo* after 4 days using a stereomicroscope (×1·2 objective, ×20 ocular). The number of vessels traversing a 1 mm annulus about the discs were counted, provided that they formed an angle of less than 45° with a line radiating from the centre of the disc. A vessel branching within the annulus was counted as one vessel. The results were evaluated statistically with Student's *t*-test.

Histological studies

The membranes were then fixed in phosphate-buffered formalin, excised, photographed and processed for histological examination. Specimens from both controls (DMEM dialysate and Swiss 3T3-conditioned medium) and experimental groups were examined 24, 48, 72 and 96 h after application of media for changes in the ectodermal epithelium, mesenchyme and vasculature as well as leukocytic infiltration.

To determine whether there was any difference in ectodermal epithelial hyperplasia in

TABLE 2. Vascular responses to control and conditioned media prepared from human epidermal keratinocytes

Medium		Number of cells/ml medium	Vascular index (mean ± s.d.)
DMEM dialysate	(11)	—	26·1 ± 8·0
Swiss 3T3	(17)	$1·5 \times 10^{6}$	28·4 ± 6·6
R strain	(18)	$2·19 \times 10^{6}$	40·2 ± 10·7††
W strain	(17)	$1·53 \times 10^{6}$	36·1 ± 7·4†
T strain	(17)	$4·35 \times 10^{6}$	44·3 ± 8·4††**
T strain (heated)	(17)	$4·55 \times 10^{6}$	30·1 ± 6·6

Number in each group given in parentheses.
† $P < 0·01$ versus controls.
†† $P < 0·001$ versus controls.
** $P < 0·001$ versus heat-treated T strain.

experimental groups versus controls, which could account for increased angiogenesis, epithelial hyperplasia was quantitated in histological sections. Briefly, using an ocular micrometer, we measured the greatest thickness of hyperplasia in each section, vertically downward from the Millipore disc (at × 200 magnification). Specimens treated with DMEM dialysate (controls) and conditioned media R and T were examined. The data were analysed with Wilcoxon's two-sample test.

RESULTS

Vascular responses

As noted in Table 2, media from all three strains of keratinocytes produced significant stimulation of vascular growth (Fig. 1) compared with the controls (Figs 2 and 3). The value of the vascular response appeared to correlate directly with the cell numbers in each culture. The T strain with $4{\cdot}35 \times 10^6$ cells/ml of medium was associated with the greatest vascular index $44{\cdot}3 \pm 8{\cdot}4$ ($P < 0{\cdot}001$). On the other hand, the W strain with $1{\cdot}53 \times 10^6$ cells/ml of medium generated the smallest response $36{\cdot}1 \pm 7{\cdot}4$ ($P < 0{\cdot}01$). Heat treatment of the T-strain medium resulted in appreciable loss of activity with a vascular index of $30{\cdot}1 \pm 6{\cdot}6$, not significantly different from controls.

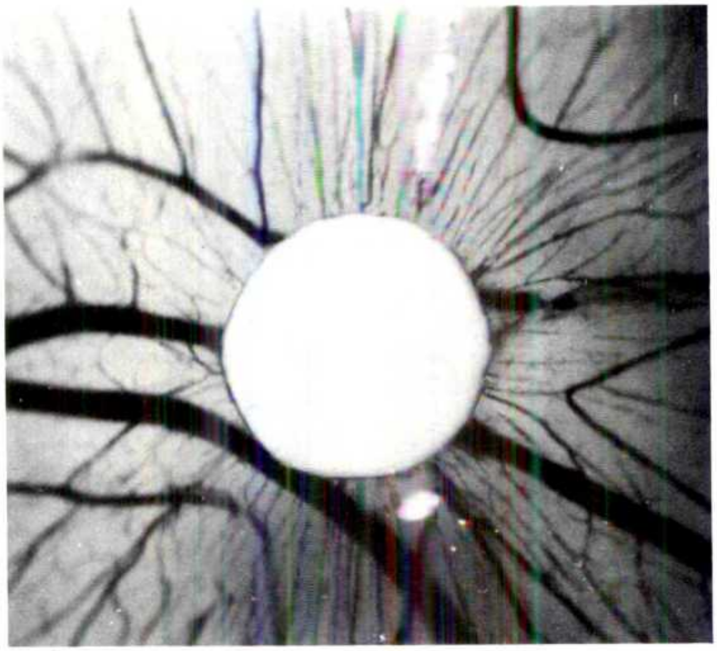

FIGURE 1. Vascular response (vascular index = 50) of the CAM to a 2-mm Millipore filter disc saturated with conditioned media prepared from human epidermal keratinocyte strain R (Table 1), after 4 days. The vascular response has resulted from hypertrophy and elongation of existing vasculature, as well as sprouting and ingrowth of new vessels (original magnification × 4).

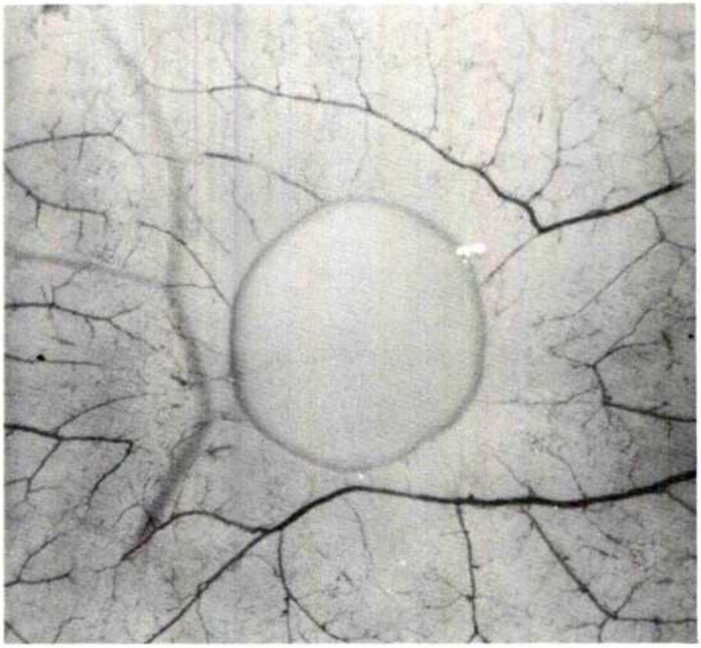

FIGURE 2. Vascular response (vascular index = 15) of the CAM to a 2-mm Millipore filter disc saturated with DMEM only, after 4 days. The vascular response is minimal (original magnification × 4).

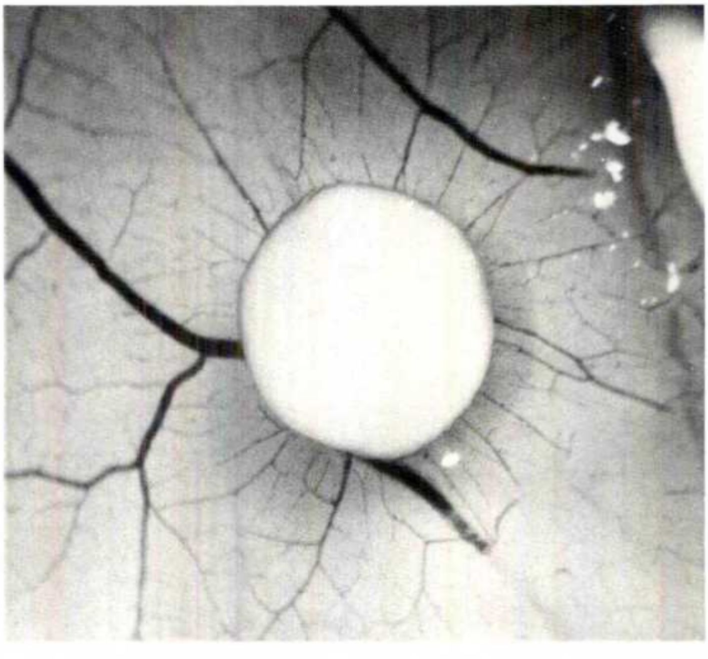

FIGURE 3. Vascular response (vascular index = 20) to a 2-mm Millipore filter disc saturated with conditioned media prepared from 3T3 cells, after 4 days. Again the reaction is minimal (original magnification × 4).

TABLE 3. Histological changes in the CAM after application of control and conditioned media

Time after application of medium	DMEM dialysate	Swiss 3T3 conditioned medium	Keratinocyte conditioned medium
Day 11 (24 h)			
Ectodermal epithelium	Minimal hyperplasia	Minimal hyperplasia	Minimal hyperplasia
Mesenchyme	Minimal hyperplasia	Minimal hyperplasia	Minimal hyperplasia
Vascularity	No significant change	No significant change	No significant change
Leukocytic infiltration	Negligible	Negligible	Negligible
Day 12 (48 h)			
Ectodermal epithelium	Hyperplasia	Hyperplasia	Hyperplasia
Mesenchyme	Hyperplasia	Hyperplasia	Hyperplasia
Vascularity	No significant change	No significant change	(?) increased
Leukocytic infiltration	Negligible	Negligible	Negligible
Day 13 (72 h)			
Ectodermal epithelium	Hyperplasia	Hyperplasia	Hyperplasia
Mesenchyme	Hyperplasia	Hyperplasia	Hyperplasia
Vascularity	Increased	Increased	Increased
Leukocytic infiltration	Negligible	Negligible	Negligible
Day 14 (96 h)			
Ectodermal epithelium	Hyperplasia	Hyperplasia	Hyperplasia
Mesenchyme	Hyperplasia	Hyperplasia	Hyperplasia
Vascularity	Increased	Increased	Increased
Leucocytic infiltration	Negligible	Negligible	Negligible

TABLE 4. Quantitation of ectodermal epithelial hyperplasia in the CAM after 4 days

Medium	Thickness of epithelium (μm) (Mean ± s.d.)
DMEM dialysate (7)	281·1 ± 131·5
R strain (10)	274·5 ± 180·4*
T strain (10)	286·5 ± 154·2*

Number in each group given in parentheses.

* N.S. ($P > 0·05$) versus controls.

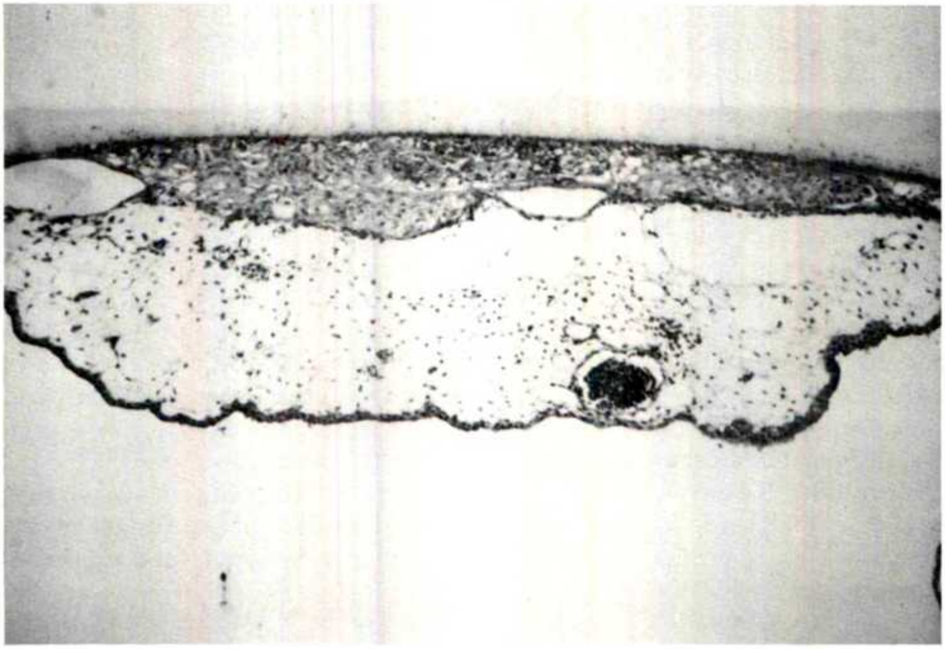

FIGURE 4. The response of the CAM after 4 days (day 14) to a Millipore filter disc saturated with keratinocyte-conditioned medium from strain R. There is both ectodermal epithelial and mesenchymal hyperplasia. There are virtually no inflammatory changes. (H & E, × 75.)

Histological results

Serial histological examination did not reveal any change in the experimental groups compared with the controls which could account for the increase in vessel growth observed (Table 3). As noted in Table 3, histological findings were very similar in all groups. There was essentially no leukocytic infiltration at any stage in any group after application of Millipore discs. Progressive development of ectodermal epithelial and mesenchymal hyperplasia occurred in all the groups (Fig. 4). Hyperplasia from keratinocyte-conditioned media was comparable to that arising in controls. The only difference was the impression that increased numbers of vessels were present beneath the discs in the experimental groups versus controls and that this increased vascularity may have developed earlier than in controls.

Quantitation of ectodermal epithelial hyperplasia (Table 4) did not indicate any significant difference in specimens treated with conditioned media from two different strains of keratinocytes when compared with controls. Despite considerable variation in each group, the results confirmed the overall impression noted on microscopy.

DISCUSSION

These studies have extended previous work supporting the notion that epidermis may promote new blood vessel growth. In contradistinction to the hamster cheek pouch studies already alluded to (Wolf & Harrison, 1973), there was no evidence for a role of inflammation in mediating angiogenesis in the present experiments. While a few leukocytes were occasionally seen in histological sections at 24 and 48 h in all groups, this number was judged to be well below the threshold needed to initiate angiogenesis (Gospodarowicz, Bialecki & Thakral, 1979). The possibility that increased vessel growth in experimental groups was secondary to a greater degree of ectodermal epithelial hyperplasia was also excluded since histological examination and quantitation showed comparable hyperplasia in all groups. Therefore, we were able to conclude that the increased vessel growth almost certainly occurred in response to the keratinocyte conditioned media rather than to other phenomena. Another bit of evidence supporting this conclusion was that the value of the vascular index correlated directly with cell numbers in the culture, a relationship also demonstrated by Klagsbrun *et al.* (1976).

The relationship of this substance to other angiogenic factors produced by tumours, leukocytes, retina and embryonic tissue as well as the epidermal factor isolated by Wolf & Harrison (1973) is intriguing but obviously remains to be elucidated. The fact that most of these substances have initially been characterized as nondialysable (mol. wt. > 10,000), heat labile, and probably proteinaceous (Wolf & Harrison, 1973; Klagsbrun *et al.*, 1976; Glaser *et al.*, 1980) has suggested possible kinship. In addition, since all of these tissues or cells share the capacity for high rates of aerobic glycolysis (Ramaiah, 1974), the possibility of a common metabolic basis may be relevant to the production of an angiogenic activity. To our knowledge, this metabolic pattern has not been previously considered by other investigators and deserves further attention.

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Analysis of the non-ionic detergent-soluble lipids of human stratum corneum*

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SUMMARY

Mechanical stimulation of the skin surface in the presence of non-ionic detergent (Triton X-100)-containing buffers has been used to prepare isolated corneocytes and non-ionic detergent-soluble (NIDS) material from human stratum corneum. Analysis of the major lipid classes present in the corneocytes and NIDS material has been performed by thin layer chromatography with flame ionization detection and quantitation based on the introduction of an internal standard. Results indicate that the NIDS lipid represents a disproportionately large amount of the total stratum corneum lipid and that it differs in composition from the corneocyte lipid, there being a greater proportion of the cholesteryl ester fraction in the corneocytes. Both these findings support the 'brick wall' model of the stratum corneum and provide further evidence that the NIDS material is intercellular in nature.

Chemically the stratum corneum is a tough, relatively inert structure which requires vigorous solubilization procedures. Reagents such as 8 M urea with mercaptoethanol are frequently used, principally to solubilize the keratin components. This approach is of little value in studying the intercellular space or corneocyte surface as these represent but a small fraction of material present. A novel approach to this problem has been the combined use of mechanical stimulation and buffers containing non-ionic detergent (Triton X-100) (King, Dykes & Marks, 1982). Corneocytes released by this procedure are essentially intact as judged by light and electron microscopy (Barton *et al.*, 1980). The outer membrane of the corneocyte is clearly seen, suggesting that the supernatant is composed to a large extent of intercellular and cell surface material. Indeed, immunolocalization studies with antisera raised to the non-ionic detergent-soluble (NIDS) protein fraction demonstrate localization of NIDS antigens to the corneocyte surface (King *et al.*, 1982), thus supporting this hypothesis.

Recent studies (Elias *et al.*, 1979; Nemanic & Elias, 1980; Smith *et al.*, 1982) have emphasized the importance of lipid material in corneocyte adhesion and transport of material across the

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stratum corneum. Lipid analysis of a newborn rat stratum corneum membrane preparation (Grayson & Elias, 1982) together with freeze fracture studies (Elias, Goerke & Friend, 1977) led Elias *et al.* (1983) to propose a two-compartment model for the stratum corneum. In this so-called 'brick wall' model, corneocytes containing relatively little lipid are surrounded by a lipid-rich domain. If such a model were valid for human stratum corneum and the intercellular lipids were important for desquamation then we might make the following predictions:

(1) that the NIDS lipid fraction represents a disproportionate amount of the total stratum corneum lipid,
(2) that the NIDS lipid fraction differs in some way from the lipid present in the corneocyte.

Here we wish to report the results of lipid analysis of NIDS material and corneocytes released from human stratum corneum *in vivo* together with data for stratum corneum. Analysis was performed by thin layer chromatography with flame ionization detection and quantitation based on the introduction of an internal standard (Bradley, Richards & Thomas, 1979). The technique of flame ionization applied to thin layer chromatography on coated quartz rods is described in detail in a recent review (Ackman, 1981).

METHODS

Analar solvents and Triton X-100 of scintillation grade were obtained from BDH Ltd. (Poole, U.K.). Cholesterol, cholesteryl oleate, squalene, ceramide, triolein, oleic acid and silica gel chromatographic grade 100–200 mesh were from Sigma Chemical Company Ltd. (Poole, U.K.). Umbelliferyl heptadecanoate was a gift from Dr D.Bradley. Millipore filters (0·22 μm) were obtained from Millipore Ltd. (Harrow, U.K.). Chromarods, type S (Iatron Laboratories Inc.) and the Iatroscan TH-10 Analyser (Iatron Laboratories Inc.) were obtained from Trivector Scientific Ltd. (Sandy, U.K.).

Collection of non-ionic detergent-soluble material, corneocytes and stratum corneum

Individual samples were collected from the forearms of twelve normal volunteers with no previous history of skin disease. In order to effectively eliminate contamination from sebum, bacteria and other skin surface debris, a three-stage washing procedure was introduced. The forearm was first washed gently with soap and water, wiped with a tissue soaked in chlorhexidine gluconate (1 in 1000) and then swabbed with 70% isopropyl alcohol. After allowing the arms to air-dry the flexor aspects were vigorously scrubbed for 5 to 10 minutes in a dish containing 100 ml sterile 0·002% Triton X-100, 0·05 M phosphate buffer pH 7·2. Medium-hard grade nylon tooth brushes were used for scrubbing. The corneocyte suspension obtained was then centrifuged at 2000 ***g*** for 20 minutes. The resulting supernatant, containing the non-ionic detergent-soluble (NIDS) material was then filtered using a 0·22 μm millipore filter. This was to ensure complete removal of corneocytes from the material to be extracted. The corneocyte pellet was resuspended in fresh phosphate buffer and the number of corneocytes estimated using a haemocytometer. Centrifugation of the suspended corneocytes was again carried out at 2000 ***g*** for 20 minutes and the supernatant discarded.

Stratum corneum was also collected from the skin surface of the forearm of twenty-one normal individuals by scraping. The skin surface was cleaned as above, any hairs present being first removed by gentle dry shaving with a scalpel blade. The skin surface was then firmly scraped with a scalpel blade to remove fragments of stratum corneum.

Extraction of sample lipids

A known amount (usually 60 μg in 3 μl methanol) of the internal standard, umbelliferyl heptadecanoate, was added to the supernatant fraction containing the NIDS material. To this an equal volume (100 ml) of chloroform:methanol (2:1) was added and the mixture shaken vigorously. Forty ml of 0·1 M KCl was then added and the mixture again shaken vigorously. After 10–15 minutes the chloroform layer was removed, washed twice with Folch upper phase (FUP) (chloroform:methanol:water, 3:48:47) and dried using anhydrous sodium sulphate. The chloroform extract was then concentrated by rotary evaporation and subjected to column chromatography on silica gel to remove Triton (see below). The corneocyte pellet was resuspended in buffer containing Triton, the internal standard added and the suspension homogenized in a glass–glass homogenizer. Four ml of chloroform:methanol (2:1) was added and the sample homogenized again. This was followed by the addition of 1·2 ml 0·1 M KCl and a third brief homogenization. The slurry was then filtered through glass wool and centrifuged at 100 ***g*** × 5 minutes. The chloroform layer was washed once with FUP, dried with anhydrous sodium sulphate, concentrated and subjected to column chromatography (see below). Stratum corneum was extracted in two different ways: (1) as a suspension of 1 to 5 mg in 2 ml of phosphate buffer containing Triton, thus paralleling the corneocyte extraction, (2) directly, 1 to 5 mg being extracted in 4 ml chloroform: methanol (2:1). Subsequent steps were as for corneocytes except that when no Triton was present the column chromatography was omitted.

Removal of Triton X-100 by column chromatography

Columns (5 × 90 mm) of silica gel Type 1 (100–200 mesh) were poured and equilibrated with acetone. The solvent was changed to chloroform and after equilibration the samples applied. The first 25 ml eluting from the column was collected, evaporated and then analysed by thin layer chromatography on chromarods. Changing the solvent to acetone led to elution of the Triton and allowed re-use of the column.

The performance of the columns was monitored both by thin layer chromatography and by measuring the recoveries of a standard consisting of 300 μg each of ceramide, umbelliferyl heptadecanoate, cholesterol, triolein, oleic acid, cholesteryl oleate and squalene. The lipid standard plus Triton X-100 (200 μg) in chloroform was applied to the column. Using chloroform as eluant, 3 × 5 ml fractions were collected. These fractions were concentrated by rotary evaporation and analysed by thin layer chromatography on 20 × 20 cm silica gel 60 plates (Merck 5715). Plates were developed in three stages: (1) isopropyl alcohol (8 cm), (ii) hexane:2-methyl propan-1-ol:acetic acid, 100:3:1 (18 cm), (iii) methanol:chloroform, 2:1 (3 cm). Spots were visualized by exposure to iodine vapour.

In the recovery experiments the complete lipid standard was used without Triton. This was necessary as Triton interfered with chromatography on the chromarods and did not enable estimation of all the lipid standards. The lipid standard was applied to the column and the first 25 ml of eluate collected, concentrated by rotary evaporation and analysed using the Iatroscan system (see below).

Thin layer chromatography followed by flame ionization detection

Samples (2–3 μl) were applied at a point 1·5 cm from the end of the chromarods. These were then subjected to a two-stage development in conventional thin layer chromatography tanks. First development (10 cm) was in pet. ether (60–80):acetic acid (100:1·25). After air-drying the rods were developed in hexane:2-methyl propan-1-ol:acetic acid (100:3·5:1·4) for a distance of 3·5 cm and then air dried in an oven at 100°C for 5 minutes. The chromarods were scanned through the flame ionization detector at 2·3 sec/cm; hydrogen flow rate = 180 ml/min; air flow rate = 2000 ml/min. The analogue output from the analyser was integrated using a CRS 308 digital integrator (Infotranics UK Ltd., Stone, U.K.) and recorded on a chart recorder operating at 100 mV full scale deflection. Samples were always analysed in parallel with complete lipid standards to enable quantitation. Analyses were always performed within 30 hours of collecting the samples and when required extracts were stored in chloroform at −20°C overnight.

RESULTS

Removal of Triton X-100 by column chromatography

The removal of Triton X-100 from the lipid standard is demonstrated in Fig. 1 in which an iodine-stained chromatography plate is shown. Triton X-100 is absent in the first three chloroform fractions (samples 3, 4, 5) showing that it is retained by the column. Changing the solvent to acetone results in elution of the Triton (samples 6, 7, 8). Some lipid is retained by the column and eluted with acetone, e.g. in sample 8. However, recovery experiments where the amount recovered was estimated using the Iatroscan, suggested that this was likely to be a very small percentage of the total lipid applied (Table 1). No correction factors were therefore used in calculating the final results.

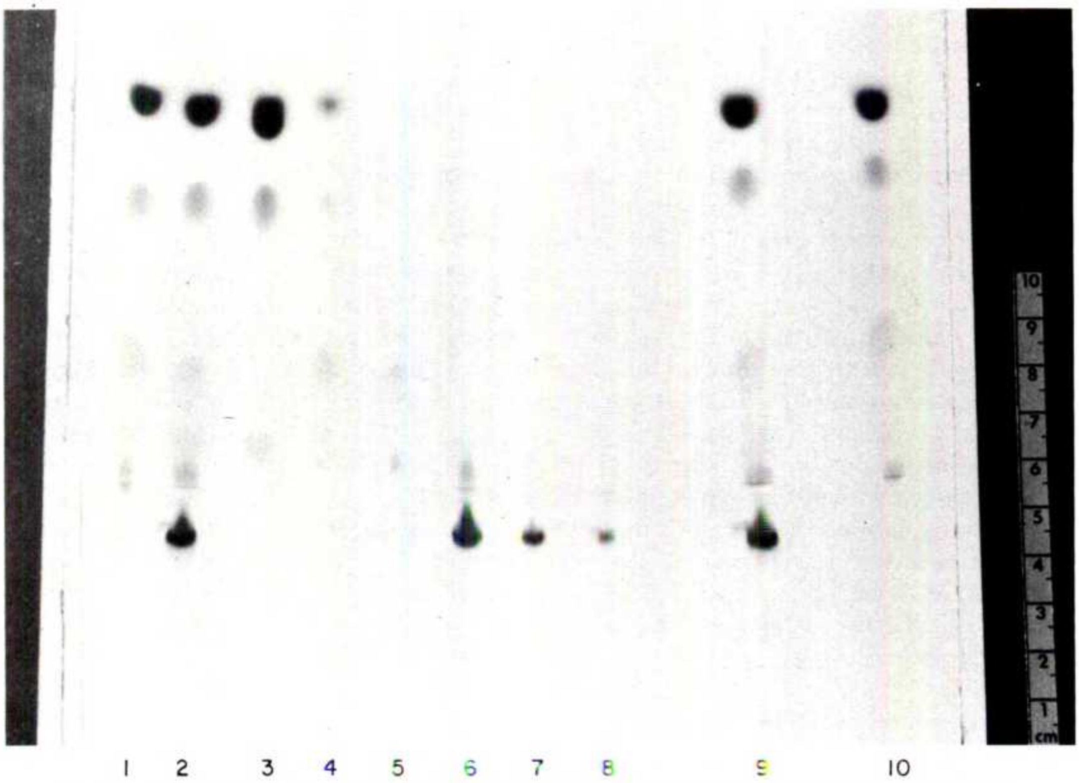

FIGURE 1. Thin layer chromatographic plate demonstrating the removal of Triton from lipid standards. Spots were visualized by iodine vapour. Samples 1, 10: complete lipid standard. Samples 2, 9: complete lipid standard plus Triton. Samples 3, 4, 5: 1st, 2nd and 3rd 5-ml chloroform fractions. Samples 6, 7, 8: 1st 2nd and 3rd 5-ml acetone fractions.

TABLE 1. Recoveries of lipid standards during column chromatography on silica gel

Lipid standard	Percentage of applied lipid recovered	
	Experiment 1	Experiment 2
Ceramide	93	104
Umbelliferyl heptadecanoate	107	135
Cholesterol	89	102
Triolein	106	127
Oleic acid	98	106
Cholesteryl oleate	73	85
Squalene	78	88
Mean ± standard deviation	92 ± 13·1	106·7 ± 18·5

Chromatography of lipid standards

Figure 2 demonstrates the resolution and detection of a mixture of lipid standards (2 μg of each) using the Iatroscan system. Each standard is resolved as a definite peak and is easily detected at this low level. This system is therefore capable of (a) resolving and measuring the six major classes of lipid compounds known to be present in the outermost layers of the stratum corneum (Gray *et al.*, 1982; Lampe *et al.*, 1983) and (b) measuring the small amounts of lipid extracted from individual samples. It does not however permit differentation of, for examples, squalene

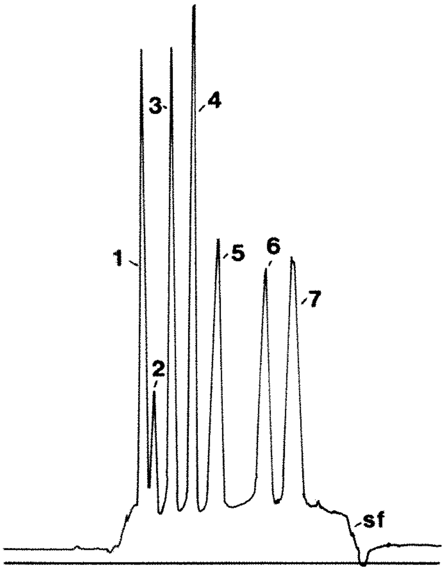

FIGURE 2. Chromatogram of standard lipid mixture developed in pet. ether (60–80): acetic acid (100: 1·25) followed by hexane: 2-methyl propan-1-ol: acetic acid (100: 3·5: 1·4). The lipid standard consisted of 2 μg of each of the following: ceramide (1), umbelliferyl heptadecanoate (2), cholesterol (3), triolein (4), oleic acid (5), cholesteryl oleate (6), and squalene (7), sf = solvent front.

and substances such as alkanes which may co-migrate with squalene in this system. The term 'lipid fraction' will be used subsequently to mean those substances co-migrating with the lipid standard and forming a peak indistinguishable from the lipid standard in this system.

Lipid analysis of NIDS lipid and corneocyte lipid

Analysis of the NIDS and corneocyte lipid of normal human stratum corneum is presented in Table 2. The results are for material removed from the forearms of twelve normal volunteers and are expressed as μg lipid per 10^6 corneocytes released and as a percentage by weight of total lipids recovered.

TABLE 2. Analysis of the non-ionic-detergent-soluble (NIDS) lipid and corneocyte lipid of human stratum corneum

	μg lipid per 10^6 corneocytes		Percentage (by weight) of total lipids	
Lipid fraction	NIDS material	Corneocytes	NIDS material	Corneocytes
SQ	11·9 ± 10·3*	15·3 ± 10·7	10·4 ± 4·0*	10·2 ± 3·6
CE	7·9 ± 8·3**	22·7 ± 21·8	5·4 ± 3·3***	13·7 ± 5·8
FFA	42·7 ± 37·1	44·4 ± 39·2	33·9 ± 12·3	29·9 ± 12·2
TG	34·0 ± 27·4	44·3 ± 33·3	26·8 ± 6·7	29·4 ± 10·7
CHL	7·3 ± 9·5	4·8 ± 4·1	5·9 ± 7·2	3·6 ± 2·5
CER	16·4 ± 12·0	16·6 ± 9·3	17·7 ± 11·5	13·2 ± 6·5
Total	120·2	148·2		

* Mean ± standard deviation (n = 12).

** Significantly lower than for corneocytes, $P < 0{\cdot}05$ (Student's *t*-test, Wilcoxon matched pairs signed rank test).

*** Significantly lower than for corneocytes, $P < 0{\cdot}001$ (Student's *t*-test).

There was little variation in composition between the NIDS lipid and corneocyte lipid. The only significant difference was observed in the cholesteryl ester fraction where a decrease was noted in the NIDS lipid. This was significant at $P < 0{\cdot}05$ when expressed as μg lipid per 10^6 corneocytes and $P < 0{\cdot}001$ when expressed as a percentage. The wide standard deviations noted were in part due to variation in the total amount of lipid released per individual (range 50–500 μg per 10^6 corneocytes). Hence when expressed as percentage the standard deviations became smaller and the difference in cholesteryl ester fraction statistically more significant.

The totals for the six lipid fractions gave values of 120·2 μg and 148·1 μg lipid per 10^6 corneocytes for the NIDS material and corneocytes respectively.

Lipid analysis of stratum corneum

A comparison of the lipid composition of corneocytes and stratum corneum is presented in Fig. 3 where the percentage distribution by weight for the six lipid fractions is presented. The samples of stratum corneum were removed from the forearms of eleven normal subjects and extracted in a manner analogous to the corneocytes, that is as a suspension in Triton X-100

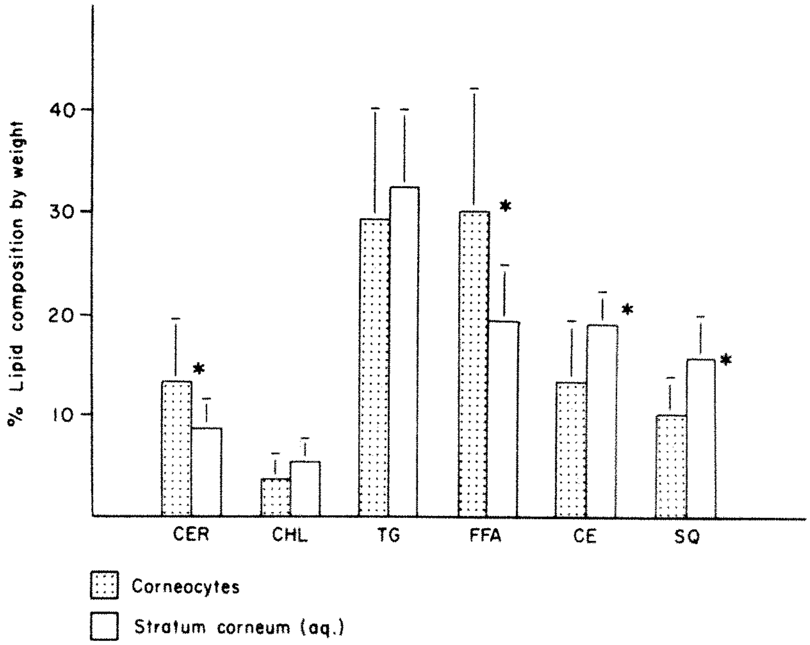

FIGURE 3. Comparison of the lipid composition of corneocytes and stratum corneum (aqueous extraction). *Statistically significant difference, Student's *t*-test. (Ceramide $P < 0{\cdot}05$; cholesteryl ester and free fatty acid $P < 0{\cdot}02$; squalene $P < 0{\cdot}01$.)

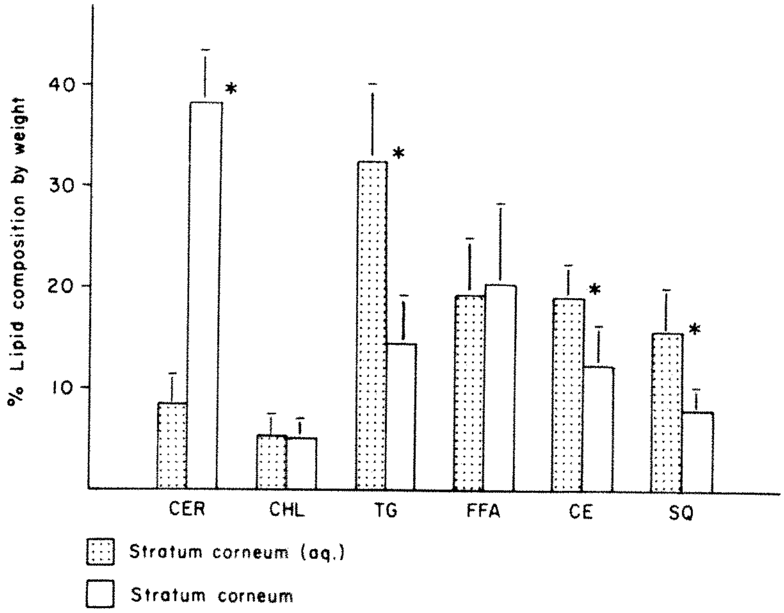

FIGURE 4. Comparison of the lipid composition of human stratum corneum extracted directly with chloroform: methanol or after suspension in Triton containing phosphate buffer (aqueous extraction). *Statistically significant difference, Student's *t*-test. (All differences: $P < 0{\cdot}001$.)

TABLE 3. Comparison of the lipid composition of human stratum corneum using different extraction methods

Lipid fraction	µg lipid per mg stratum corneum	
	Normal extraction	'Aqueous' extraction
SQ	5·3 ± 2·7*	11·9 ± 7·2**
CE	8·2 ± 3·4	14·3 ± 8·6
FFA	13·5 ± 5·4	13·5 ± 7·9
TG	9·8 ± 4·7	23·3 ± 11·7
CHL	3·2 ± 1·2	3·5 ± 2·1
CER	25·4 ± 8·8	6·1 ± 4·0

* Mean ± standard deviation for n = 10 normal subjects.

** Mean ± standard deviation for n = 11 normal subjects.

containing phosphate buffer (aqueous extraction). Although the overall lipid composition was similar, statistically significant differences were observed in the ceramide, free fatty acid, cholesteryl ester and squalene fractions. These differences were still apparent if the NIDS lipid and corneocyte lipid data were combined and then compared with data for the stratum corneum (data not shown).

Comparison of the lipid composition of stratum corneum (aqueous extraction) and stratum corneum extracted directly with chloroform : methanol (2 : 1) in a conventional manner is presented in Fig. 4. Large differences were observed in the ceramide and triglyceride fractions with smaller but statistically significant differences in the cholesteryl ester and squalene fractions. These differences are also apparent when the data is expressed as µg per mg stratum corneum (Table 3).

DISCUSSION

It has been estimated that the intercellular space comprises some 10–30% of the total volume of the stratum corneum (Grayson & Elias, 1982; Plewig *et al.*, 1983). The finding of almost equal amounts of lipid in the NIDS fraction and corneocytes (Table 2) would suggest that either the detergent is extracting large amounts of intracellular lipid or that the intercellular space is a lipid-rich domain. In view of the recent evidence in favour of a lipid-rich intercellular zone (Elias *et al.*, 1983) it would seem most likely that the NIDS material is intercellular in origin. If this were indeed the case, and intercellular lipid played a role in desquamation and transport of material across the stratum corneum, then the composition of the NIDS lipid might be expected to differ from that of the corneocytes. Comparison of the various lipid fractions (Table 2) did indicate some differences in composition. The most significant difference was found in the cholesteryl ester fraction where a decrease was found in the NIDS lipid. This difference was apparent both when the results were expressed as µg per 10^6 corneocytes and as percentage by weight of total lipids.

The significance of there being more cholesteryl ester in the corneocyte and its possible role in the function of the stratum corneum is uncertain. The importance of sterols and sterol esterification in the process of keratinization has long been recognized (Yardley, 1969; Yardley & Summerley, 1981).

Recent evidence suggests an accumulation of sterol/wax esters in the outermost layers of the stratum corneum (Lampe, Williams & Elias, 1983). However, the exact mechanism whereby changes in sterols and sterol esters produce abnormal desquamation is not known. One possibility is interaction of sterols with phospholipid metabolism. For example, the co-ordination of cholesterol and phosphatidylcholine biosynthesis has recently been demonstrated *in vitro* (Cornell & Goldfine, 1983). In addition the formation of cholesteryl esters via the lecithin: cholesteryl transferase reaction has also been described (Glomset, 1968). To what extent these pathways contribute to keratinization is not clear but the relative accumulation of cholesteryl ester in the corneocyte may well be due to lecithin: cholesteryl transferase type reactions. If the accumulation of cholesteryl ester in the corneocyte were important in the process of desquamation then inhibition of sterol synthesis might lead to a disordered keratinization via inhibition of the lecithin: cholesteryl tranferase reaction. These observations must remain speculative until further information is available concerning the biosynthesis of cholesteryl esters in the stratum corneum.

Comparison of the lipid composition of corneocytes with stratum corneum (Fig. 3) indicated differences in composition. These persisted even when a comparison was made between stratum corneum and the NIDS material and corneocytes combined. One possible explanation for this discrepancy is that the extraction of lipids is altered by prior dispersion of the stratum corneum. Thus although an attempt was made to duplicate the corneocyte extraction procedure, i.e. by extracting/homogenizing the stratum corneum in Triton containing phosphate buffer, the conditions were sufficiently different to lead to altered lipid composition.

That the extraction conditions are important in terms of lipid composition is illustrated in Fig. 4, where large differences were observed when stratum corneum was extracted by two different methods. In the presence of an aqueous phase it would appear that a greater percentage of non-polar (e.g. squalene) rather than polar (e.g. ceramide) lipids are extracted into the lipid phase. These differences make it difficult to compare these data with other work, e.g. that of Lampe *et al.* (1983). In addition the system described here does not differentiate between, for example, squalene and alkanes which are known to exist in normal stratum corneum (Williams & Elias, 1983). Nevertheless with the exception of the cholesterol fraction, our results for the stratum corneum are similar to the values given for outer human stratum corneum (Lampe *et al.*, 1983). Values in the order of 15–20% for cholesterol have been given for human (Lampe *et al.*, 1983), pig (Gray *et al.*, 1982) and neonatal mouse (Grayson & Elias, 1982) stratum corneum. Our values are much lower (see Fig. 4) for both methods of extraction. However the absolute amounts of lipid in the cholesterol fraction (see Table 3) are not dissimilar to the values given by Schmidt *et al.* (1977) using a biochemical method of assay. Further work is needed to resolve these discrepancies.

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The effect of corticosteroids, dapsone and gold upon plasminogen activator synthesis and secretion by human epidermal cells cultured with pemphigus antibody

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SUMMARY

Human epidermal cells were cultured with pemphigus antibody in the presence of hydrocortisone, methylprednisolone sodium succinate, dapsone and gold. Hydrocortisone and methylprednisolone reduced the production of the enzyme plasminogen activator but dapsone and gold had no effect. These results support the hypothesis that corticosteroids have dual effects on pemphigus through inhibition of plasminogen activator production by epidermal cells and suppression of pemphigus antibody production by B lymphocytes.

Beutner & Jordan (1964) demonstrated that serum from pemphigus patients contains autoantibodies which bind to the intercellular substance of skin and mucosa. Michel & Ko (1974) reported that incubation of pemphigus antibody with organ cultures of normal human skin resulted in histological changes identical to those seen in biopsies of skin from pemphigus patients. Schiltz & Michel (1976) showed that the IgG fraction of pemphigus serum was responsible for loss of cellular adhesion in a complement-independent manner. Farb, Dykes & Lazarus (1978) suggested that a proteinase was responsible for the acantholysis found in pemphigus. Incubation of cultured mouse epidermal cells with pemphigus antibodies resulted in detachment of cells from the culture vessel. Release of epidermal cells was blocked by the addition of the proteinase inhibitors, soybean trypsin inhibitor and α_2-macroglobulin. In a recent study, we demonstrated that binding of pemphigus antibody to the surface of epidermal cells stimulates synthesis and secretion of plasminogen activator (Hashimoto *et al.*, 1983); this

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enzyme activates plasminogen to plasmin and it is the plasmin which is responsible for loss of cellular adhesion and acantholysis.

It is important to investigate whether drugs used in the treatment of pemphigus possess direct effects on synthesis and secretion of plasminogen activator by cultured epidermal cells incubated with pemphigus antibodies or not. In the present study, we report the effect of hydrocortisone, methylprednisolone, dapsone and gold salts on production of plasminogen activator by epidermal cells induced with pemphigus antibody.

METHODS

Human epidermal cells from the foreskin of human neonates were cultured using a modification of the technique of Rheinwald and Green (Rheinwald & Green, 1975). After reaching confluence the cells were incubated overnight with DME containing 20% FCS and no other additives. The cultures were then incubated with DME (1 ml/well) containing pemphigus IgG (2 mg/ml). Hydrocortisone (Calbiochem-Behring Co.), methylprednisolone sodium succinate (Upjohn Co.), gold sodium thiomalate (Merck Sharp and Dohme Co.) and dapsone (Sigma Co.) were added to the medium at concentrations of 10^{-5} M to 10^{-8} M. Twenty-four hours later this conditioned medium was harvested and assayed for extracellular plasminogen activator (PA) activity. The cells were then washed three times with phosphate-buffered saline and extracted with 0·5% Triton X-100 in 0·01 M Tris–HCl, pH 7·8 for cell-associated PA activity. The pemphigus IgG employed was purified from an 18-year-old boy with pemphigus foliaceus, by lysine-Sepharose and protein A-Sepharose chromatography as described previously (Hashimoto *et al.*, 1983). Plasminogen activator was assayed using multi-well culture dishes coated with [^{125}I]fibrin as previously described (Hashimoto *et al.*, 1983). PA activity in conditioned medium and cell lysates was compared with the activity of a standard preparation of human urokinase measured on the same day. Extracellular PA activity was expressed as Ploug milli-units (mU) per ml conditioned medium collected from one well of a Costar cluster 6 plate (surface area 8 cm^2). Cell-associated PA activity is expressed as Ploug milli-units per ml cell lysate extracted from one well.

RESULTS

Human epidermal cells incubated with this preparation of pemphigus IgG demonstrate an eightfold increase in plasminogen activator activity in both the culture medium and the cells.

Epidermal cells were incubated for 24 h in the presence of pemphigus foliaceus IgG and varying concentrations of hydrocortisone, methylprednisolone, dapsone and gold salts. The steroid derivatives, hydrocortisone and methylprednisolone, at 10^{-5} M inhibited about 80% of extracellular and cell-associated PA activity induced by pemphigus antibody (Fig. 1a,b). At 10^{-7} M, methylprednisolone showed a decrease of 35% and 66% in extracellular and cell-associated PA activity respectively; by contrast hydrocortisone did not inhibit plasminogen activator activity at the same concentration. These data suggest that methylprednisolone is a more potent inhibitor of PA production induced by pemphigus antibody than hydrocortisone. The effects of dapsone and gold salts are shown in Figs 1c and 1d respectively. Neither compound inhibited PA production induced by pemphigus antibody, intracellularly or extracellularly. Concentrations examined in the present study were chosen to include therapeutic doses of these four compounds. The maximum concentration of hydrocortisone in body fluids would be approximately 7 μM, assuming that 100 mg of hydrocortisone were administered systemically and the compound completely equilibrated with 40 l of body fluid

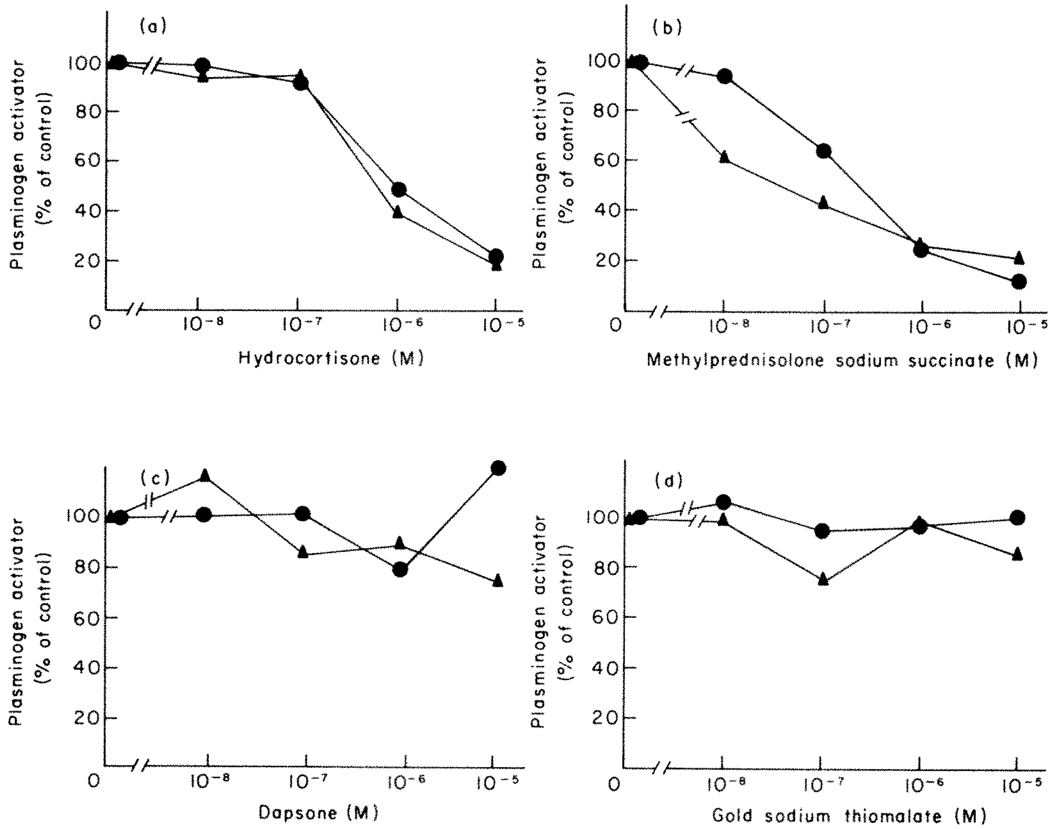

FIGURE 1. Effect of hydrocortisone, methylprednisolone, dapsone and gold salts on pemphigus-induced PA synthesis and secretion. Confluent cultures of human epidermal cells were incubated for 24 h at 37° C with purified pemphigus foliaceus IgG (2 mg/ml) in the presence of various concentrations of hydrocortisone (a), methylprednisolone (b), dapsone (c) and gold salts (d). Extracellular PA (●) and cell-associated PA (▲) was quantitated using the $[^{125}I]$fibrin plate assay.

(Schiltz, Hu & Michel, 1979). The maximum concentration of methylprednisolone would be the same as that of hydrocortisone. The actual concentrations of these two compounds in the epidermis are probably lower than this concentration, since most corticosteroids are catabolized, and/or excreted into the urine. Separd *et al.* (1976) reported that plasma concentrations of dapsone range from 0·4 to 1·2 μg/ml (1·6 μM to 4·8 μM) 24 h after oral ingestion of 100 mg of dapsone. The concentration of dapsone in the epidermis would not be higher than this concentration. Schiltz *et al.* (1979) suggested that the gold concentration in the epidermis would be 5·9 μM based on the data of Penneys, Kramer & Gottlieb, (1975).

DISCUSSION

Corticosteroid, dapsone and gold salts are effective in the management of pemphigus (Lever, 1972; Piamphongsnant, 1976; Penneys *et al.*, 1973). A postulated mode of action of these compounds is inhibition of pemphigus antibody production, since pemphigus antibody titres decrease with treatment of these reagents. We reported that production of plasminogen activator by epidermal cells following incubation with pemphigus antibody results in a localized increase in plasmin which induces loss of epidermal cohesion and acantholysis. This observation led us to determine whether these four compounds could inhibit production of plasminogen activator by epidermal cells incubated with pemphigus immunoglobulin. The present study clearly demonstrated that corticosteroids can inhibit PA production by epidermal cells, but dapsone and gold salts had no effect. We have recently used very large doses of methylprednisolone (pulse therapy) for the treatment of severe pemphigus and observed suppression of disease activity, as measured by loss of the Nikolsky sign and cessation of spontaneous blistering, within 48 h (Johnson & Lazarus, 1983). Pemphigus antibody titres often drop significantly only after 2–4 months of steroid therapy and some patients become clinically free of disease while still possessing a raised antibody titre (O'Loughlin, Goldman & Provost, 1978). This effect of corticosteroid could be explained by direct inhibition of PA production. Schiltz *et al.* (1979) reported that hydrocortisone could not prevent acantholysis in human skin explants cultured with pemphigus IgG. On the other hand, Swanson & Dahl (1980) found that methylprednisolone could prevent acantholysis in human skin explants cultured with pemphigus IgG when methylprednisolone was added 24 h before initiating incubation with pemphigus IgG. This discrepancy may be explained by the difference in inhibitory activity by the steroid derivatives and the possible need for an incubation time for inhibitor activity. Anhalt and co-workers (1983) could not inhibit experimental pemphigus in newborn mice with corticosteroids; interpretation of these experiments must await further study of the steroid responsivity of neonatal mice. The mechanism of corticosteroid inhibitory action on PA production has not been completely elucidated. Corticosteroid induces inhibitor(s) of plasminogen activator in human fibroblasts and human embryonic lung cells (Crutchley, Conanan & Maynard, 1981; Jaken, Geffen & Black, 1981). Since there are inhibitor(s) of plasminogen activator in the epidermis (Nishioka & Ryan, 1971), an increase in the amount of plasminogen activator inhibitor is one possible explanation for corticosteroid action. Dapsone and gold salts are effective forms of therapy for pemphigus but they have a long latency period beyond therapeutic efficacy. The results presented support a dual effect of corticosteroids upon pemphigus. The first is a direct effect on acantholysis through inhibition of PA production by epidermal cells, and the second is an indirect effect by suppression of pemphigus antibody production by B lymphocytes.

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Cutaneous carcinoma in psoriatic patients treated with PUVA

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SUMMARY

Eight of 216 psoriatic patients treated at The London Hospital with photochemotherapy (PUVA) have developed a total of twenty-five skin carcinomata. The type and site of tumour are discussed with special reference to the importance of PUVA compared with other risk factors. We suggest that a history of previous skin carcinoma, arsenic therapy or radiotherapy are relative contra-indications to PUVA therapy for psoriasis.

Photochemotherapy with 8-methoxypsoralen and ultraviolet A (PUVA) is an effective form of treatment for severe psoriasis (Parrish *et al.*, 1974; Swanbeck *et al.*, 1975; Wolff *et al.*, 1976; Lakshmipathi *et al.*, 1977; Melski *et al.*, 1977; Roenigk *et al.*, 1979; Henseler *et al.*, 1981). It is popular with patients because it avoids the inconvenience of topical applications. The incidence of short-term side-effects is remarkably low (Melski *et al.*, 1977) and it is clear that maintenance PUVA will keep patients in clinical remission for long periods (Henseler *et al.*, 1981). To be weighed against these established benefits of PUVA there are risks such as premature ageing of the skin and carcinogenesis, which have yet to be quantified.

In this report we describe our experience of cutaneous carcinomata in patients treated by PUVA at The London Hospital over the last 7 years.

METHODS

We have used PUVA since 1976 and treated 216 patients, 115 males and 101 females whose ages ranged from 16 to 81 years (mean 49). Eighty-nine patients are currently on either a clearing regime or maintenance therapy. Patients were asked to report the development of any unusual lesions and were carefully examined at intervals during their treatment to detect the development of tumours. Detailed enquiry was made concerning previous exposure to arsenic, radiotherapy or excessive sunlight. Biopsies taken from the patients in whom tumours developed were reviewed by two pathologists (F.C. and D.J.P.). In each case the type of tumour and its depth of invasion was recorded together with changes in the surrounding skin with special regard to keratosis, elastosis and psoriasis.

TABLE 1. Clinical details and pathological findings

Case no.	Sex	Starting age on PUVA	Duration psoriasis (years)	Risk factors	Skin type	Tumours prior to PUVA	Interval between PUVA and tumour (months)	Number of J/cm² prior to tumour	Tumours and site	Exposed (E) Unexposed (UE)
1	M	60	37	Lived in Egypt for 2 years	III	0	47	419	2 SCC (chest, back)	UE
2	F	47	8	None	III	0	58	1190	2 KA (2 arm)	E
3	M	57	22	None	III	0	40	422	1 BCC (nose)	E
4	F	62	18	None	III	1 BCC	2	66	1 KA (breast)	UE
5	M	67	46	None	III	0	48	354	1 BCC (neck)	E
6	M	69	40	Outdoor worker	III	0	26	491	1 SCC (face)	E
7	M	37	15	Azathioprine Methotrexate Hydroxyurea	III	0	39	862	2 SCC (upper leg, upper arm)	UE
8	M	68	47	X-rays Bleomycin N_2 mustard Hydroxyurea Azathioprine Arsenic Methotrexate	IV	4 BCC	21	201	8 SCC (2 back, 2 chest, 2 scrotum, 1 abdomen and 1 arm) 4 BCC (2 back, 2 legs) 3 Bowen's disease (chest, scrotum, back)	UE UE UE

RESULTS

Table 1 summarizes the details of the eight patients who developed a total of twenty-five tumours. In four patients the tumours were multiple. Of the twenty-five lesions, thirteen were squamous cell carcinomata, six basal cell carcinomata, three kerato-acanthomata and three areas of Bowen's disease. Only one patient had previous arsenic and radiotherapy. His lesions showed interesting differences compared with those of the seven other patients. All of the fifteen lesions which developed in this patient arose on skin which is not normally exposed to light. In contrast, five of the ten lesions in the other patients occurred on exposed skin.

The one patient (case 8) who had been exposed to arsenic, radiotherapy and other possible risk factors, developed multiple tumours. His history is therefore presented in detail.

Case 8: T.L.

This male patient developed severe psoriasis in 1929 when he was 21 years of age. Treatment with topical applications proved difficult and a course of superficial X-ray therapy was given to the buttocks. At the age of 61 he developed his first basal cell carcinoma. A year later he developed very severe erythrodermic psoriasis with arthropathy and this proved very difficult to control. Systemic steroids, combined with agents including azathioprine, hydroxyurea, bleomycin and topical nitrogen mustard were ineffective. Methotrexate proved effective for a time but a dose of 300 rad (cGy) of Grenz rays over 3 weeks was also required. An episode of pustular psoriasis occurred when he was aged 68 and liquor arsenicalis was prescribed. Shortly afterwards three basal cell carcinomata were found on non-exposed sites.

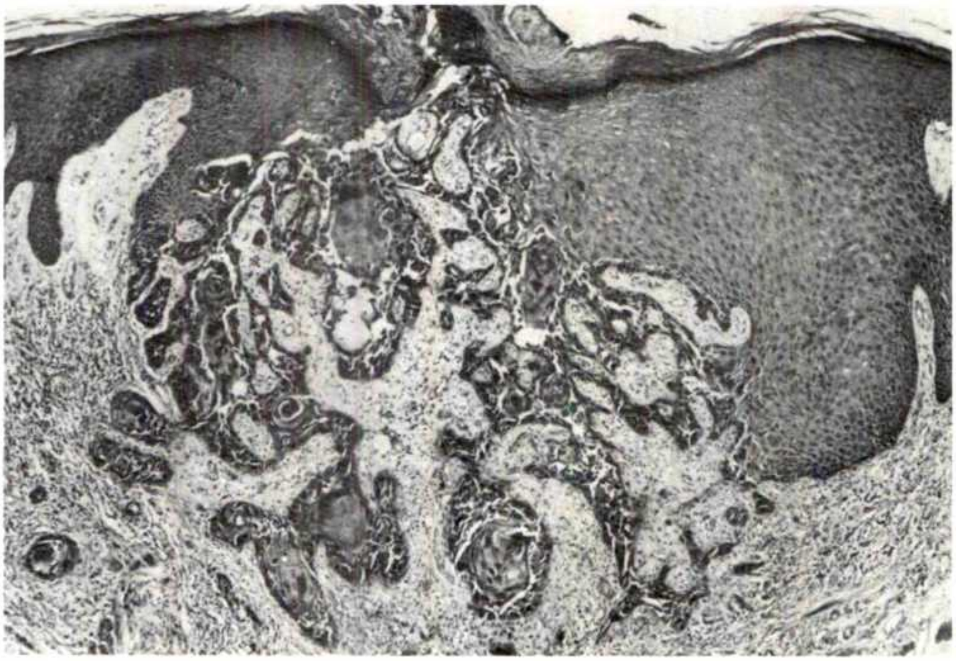

FIGURE 1. Case 8. Lesion from scrotum. Pseudo-glandular pattern of squamous cell carcinoma with irregular down growths, some showing clefting, extending to the dartos muscle. (Haematoxylin and eosin, × 10.)

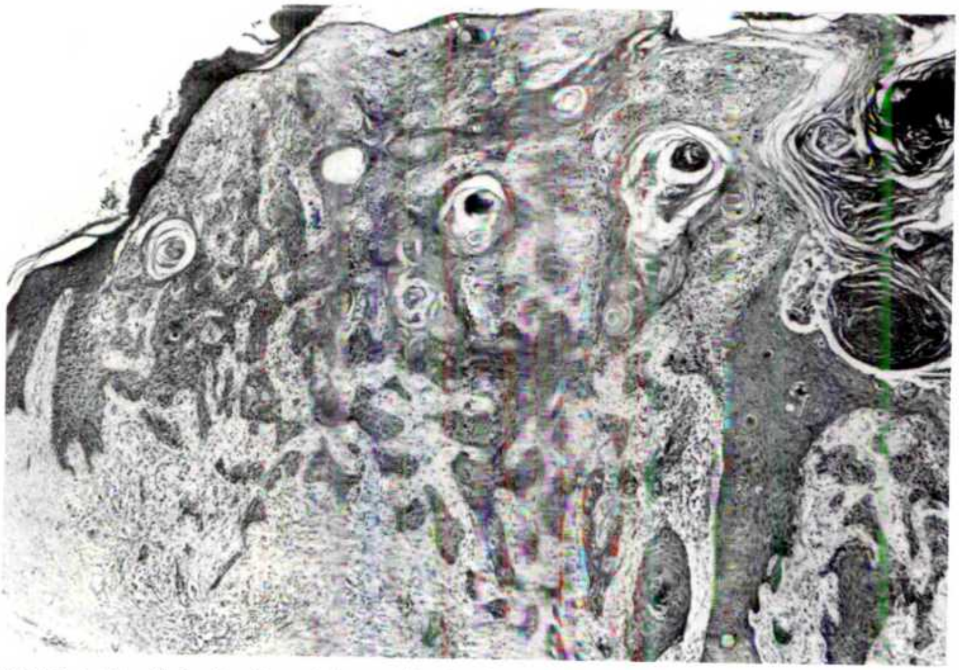

FIGURE 2. Case 8. Lesion from abdomen. Section from the edge of a keratoacanthoma-like invasive squamous cell carcinoma. (Haematoxylin and eosin, × 10.)

In 1976 when aged 68, he came under our care at The London Hospital. PUVA therapy was started and proved highly effective. All other therapy was gradually withdrawn. He had been treated for 21 months and had received 201 J/cm² UV-A when a squamous cell carcinoma appeared on his lower back and over the next five years fourteen further lesions developed (Table 1). PUVA therapy has proved the only effective therapy for this patient's disease and has therefore been continued with careful surveillance and prompt excision of the tumours.

Histopathology

The histological appearances of the tumours showed few special features. The thirteen squamous cell carcinomata were all well differentiated, three extended to the deep dermis and three invaded subcutaneous tissue. Two squamous tumours were of the pseudoglandular type, both from case 8. (Fig. 1). Five squamous tumours had the low power configuration of keratoacanthoma (cases 6 and 8) but invasion, much nuclear pleomorphism and dyskeratosis were present (Fig. 2). Six basal cell carcinomata were found; three were of adenoid type and two were multifocal (cases 3 and 8); only one had extended beyond the upper dermis.

The skin adjacent to the tumour showed keratosis in four cases. In case 8 there was keratosis associated with acantholysis, often with irregular buds of epithelium extending down from the lower epidermis.

DISCUSSION

Epidemiological surveys have shown that solar ultraviolet radiation is a major aetiological factor in the development of skin tumours (Scotto, Kopf & Urbach, 1974; Fears, Scotto &

Schneidermann, 1977) and point to the hazards to be expected from the therapeutic use of ultraviolet radiation. Experience with the use of ultraviolet light B for psoriasis has shown this to be a relatively safe long-term form of treatment (Pittelkow *et al.*, 1981). In a 25-year follow-up study of psoriatic patients treated with coal tar and UV radiation, they demonstrated no appreciable increase in the number of tumours. In a study of 305 patients treated for atopic eczema with tar and ultraviolet light over a previous 25-year period, Maughan *et al.* (1980) found that the incidence of tumours was similar to that in the general population. In addition, most of the tumours were on sun-exposed skin and eight out of eleven patients who developed carcinomata had also received X-ray therapy to these sites.

As yet, there is of course no comparable experience of PUVA, which was only introduced 10 years ago. Theoretical considerations indicate mechanisms by which PUVA might act as both a tumour initiator and tumour promoter. Psoralens are known to combine with the pyrimidine bases in DNA and are then able to cross-link the opposite strands of the DNA double helix. In experimental situations, PUVA-induced DNA damage has been produced in bacterial and mammalian cell cultures and human peripheral lymphocytes (Bridges, 1978). In hairless albino mice both 8-methoxypsoralen and 5-methoxypsoralen increase the incidence of tumours following exposure to solar simulated radiation (Young *et al.*, 1983). PUVA accelerates the development of neoplasms in patients with xeroderma pigmentosum providing evidence of its capability of inducing DNA damage in the human skin in a therapeutic situation (Reed, 1976).

It is thus not surprising that reports of skin carcinomata in PUVA-treated patients have emerged (Stern *et al.*, 1979; Tam, Van Scott & Urbach, 1979; Hoffman, Plewig & Braun-Falco, 1979; Brown, Burnett & Robinson, 1980). Unfortunately the importance of PUVA, relative to other risk factors, has been difficult to assess. Stern *et al.* (1979) reported an increased incidence of cutaneous carcinomata amongst 1373 patients treated with PUVA and noted a higher than expected proportion of squamous cell carcinomata as well as an excess of squamous cell carcinomata in areas which had not been exposed to the sun. However, 27% of patients in the series had been treated with ionizing radiation for psoriasis. Tam *et al.* (1979) reported a case of multiple lesions of Bowen's disease and squamous cell carcinoma on the penis and skin of the pubic region of a 32-year-old man undergoing PUVA treatment. However, this patient had previously received aminopterin, methotrexate, Grenz rays and hydroxyurea. Keratoacanthomata and Bowen's disease were described in PUVA-treated patients by Hoffman *et al.* (1979), but two of the four patients included had received arsenic therapy.

Hönigsmann *et al.* (1980) monitored a group of 418 patients treated with oral photochemotherapy. They concluded that the incidence of non-melanoma skin tumours in long-term PUVA patients was not different from the expected incidence in the general population, providing that there was not previous treatment with arsenic or ionizing radiation. They concluded that arsenic was the most important risk factor.

Brown *et al.* (1980) felt that PUVA might be responsible for multiple basal cell carcinomata seen on non-sun-exposed skin in two patients. However both patients had received previous radiotherapy so that the role of each of the risk factors was difficult to evaluate. In another large study, Roenigk & Caro (1981) concluded that the anatomical distribution of lesions and the ratio of basal cell carcinomata to squamous cell carcinomata was much the same as in spontaneously occurring lesions in the U.S.A. They blamed ionizing radiation rather than arsenicals as a major risk factor. Australian experience with 489 patients treated with PUVA (Lobel *et al.*, 1981) was that lesions occurred exclusively on sun-damaged skin and the authors recommended the protection of such areas from PUVA.

Our study of skin carcinomata in patients receiving psoralen photochemotherapy differs in two important respects from earlier reports. Firstly, the follow-up period was much longer, so that a tumour arising 58 months after the start of therapy is described here (case 2). In addition only one of our patients had been exposed to arsenic and ionizing radiation, and he developed multiple tumours within 21 months of onset of PUVA, suggesting a strong promoter effect.

The frequency of the use of arsenic and ionizing irradiation in the previous studies has made it difficult to quantify PUVA as carcinogenic factor. In our seven patients who had no exposure to these risk factors there was a high ratio of SCCs to BCCs (2·5 : 1) and half the tumours occurred on non-exposed skin. This differs from the pattern of naturally occurring lesions in the United Kingdom where basal cell carcinomata are the commoner tumours and exposed skin is usually affected (Sanderson & MacKie, 1979). In this regard it is notable that in our group of patients, basal cell carcinomata were the only tumours developing before the start of PUVA therapy. The occurrence of pseudo-glandular squamous cell carcinoma and squamous carcinoma of a keratoacanthoma-like type is worth noting since these tumours are often associated with sun exposure (Johnson & Helwig, 1966). These authors also mention the acantholysis noted in our case 8.

Our continuing observations in case 8 strongly support the contention that previous skin carcinomata, arsenic therapy or radiotherapy are strong risk factors and are relative contra-indications to photochemotherapy for psoriasis. The anatomical distribution and incidence of squamous cell tumours in our other patients suggest that PUVA is a significant risk factor in the development of non-melanoma skin tumours in the United Kingdom. However, no metastases have occurred in our patients so far, local treatment of tumours is easy and the patients are prepared to accept the risk in return for control of their disease.

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The relationship between linear IgA disease and benign mucous membrane pemphigoid

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SUMMARY

Nine out of thirty-four patients with linear IgA disease (LAD) had oral ulceration. Four out of seven of these patients who were examined by an ophthalmologist had changes of a cicatrising conjunctivitis indistinguishable from those of benign mucous membrane pemphigoid (BMMP). Three of these patients gave no history of ocular symptoms up to the time of examination. These findings indicate a need for oral and ophthalmological assessment in all patients with LAD.

Twenty-seven patients with a diagnosis of BMMP were also studied. Nine presented with oral symptoms alone, nine with ocular symptoms alone, seven with oral and ocular symptoms, and two with cutaneous lesions in addition to oral and ocular symptoms. All the patients were examined by the same ophthalmologist. Six of the nine patients who presented with oral symptoms alone had signs of a cicatrising conjunctivitis. Four of these six patients had the clinical pattern of erosive gingivitis which was not previously thought to be associated with a cicatrising conjunctivitis. There is a similar need, therefore, for an ophthalmological assessment in all patients presenting with oral BMMP.

Three of the twenty-seven patients with BMMP had homogeneous-linear deposits of IgA in uninvolved skin. The finding of linear IgA deposits in the skin of these patients with only mucous membrane lesions, and the finding that patients with LAD have a high incidence of oral and conjunctival lesions, raise the possibility of a common pathogenic pathway but with varying clinical expressions in these two groups.

Recent case reports have indicated that mucous membrane lesions are a feature of subepidermal bullous dermatoses other than benign mucous membrane pemphigoid (BMMP) (Marsden & Greaves, 1982; Wojnarowska & Keczkes, 1982). This study reports the high incidence of mucous membrane involvement in adults with linear IgA disease (LAD). It also examines in detail the group of disorders that comes under the label of BMMP and questions the justification for considering BMMP as a distinct entity.

METHODS

Linear IgA Disease

Thirty-four patients were included in the multi-centre study of LAD previously reported (Leonard *et al.*, 1982). All the patients had a rash characterized by spontaneous blistering, but no cutaneous scarring, and immunofluorescence showed homogeneous-linear (HL) deposits of IgA in uninvolved skin. As part of that study the referring physicians were asked to record any history of oral lesions. Seven patients from the study, who lived locally, were asked for a history of ocular symptoms and had their eyes examined by the same ophthalmologist (P.W.). Only one of these patients had oral ulceration. The average age of this subgroup of patients was 44 years (range 21–73) and the average duration of disease at the time of examination was 8 years (range 1–40). All these patients were taking a normal diet at the time of study and the rash was being treated with sulphones.

Benign mucous membrane pemphigoid

Twenty-seven patients with BMMP were studied. They were selected either by the presence of oral blistering and ulceration with histological and immunofluorescent features of BMMP (Bean *et al.*, 1979; Sneddon, 1979), or by symptoms and signs of a cicatrising conjunctivitis for which other causes had been excluded clinically. Nine patients presented with oral symptoms alone, nine with ocular symptoms alone, seven with oral and ocular symptoms and two with oral, ocular and cutaneous symptoms. None of the patients had genital lesions. There were fifteen men and twelve women, of average age 59 (range 24–85) and the average duration of disease at the time of study was 6 years (range 0·5–20). These patients were investigated as follows.

Clinical features. All patients were asked for a history of eye symptoms and were examined by the same ophthalmologist (P.W.) for signs of a cicatrising conjunctivitis. The pattern of oral involvement, when present, was noted.

Tissue biopsies. All patients with active oral blistering and ulceration had biopsies of mucosal lesions to confirm the diagnosis histologically, and perilesional tissue for immunofluorescent study. In addition, histologically uninvolved mucous membrane biopsies were taken for immunofluorescence in a further three patients, who gave a history of oral blistering and ulceration but who were in clinical remission at the time of study. Uninvolved skin biopsies were taken with a 4-mm biopsy punch in all twenty-seven patients with BMMP. The perilesional mucous membrane biopsies and the uninvolved skin biopsies were embedded in OCT medium (Lab-Tek Inc.), and snap-frozen in liquid nitrogen. Cryostat sections of the biopsies were examined for the presence of IgA, IgG, IgM and C_3 by direct immunofluorescence as described previously (Fry *et al.*, 1978).

Anti-gliadin antibodies. The presence of anti-gliadin antibodies (AGA) in the serum was tested for by the enzyme linked immunosorbent assay as described previously (Unsworth *et al.*, 1981). Sera with an AGA titre greater than 15% of the reference serum were regarded as positive and tested for class specificity.

Jejunal biopsy. A jejunal biopsy by Crosby capsule was performed wherever possible in all patients with AGA-positive sera, and in those with an HL deposit of IgA in uninvolved skin. The specimen was examined for morphological abnormality using a dissecting microscope. An intra-epithelial lymphocyte count (IELC) was performed on H & E stained sections of the biopsies (Fry *et al.*, 1974).

Circulating anti-basement membrane zone antibodies. The presence of circulating anti-basement membrane zone antibodies (ABMZA) was detected using normal human skin as the substrate (Katz, Halprin & Inderbitzen, 1969).

HLA typing. HLA typing for A and B loci was performed by the NIH microcytotoxicity test (Terasaki & McClelland, 1964).

RESULTS

Linear IgA disease

Nine out of the thirty-four patients had oral blistering and ulceration (Fig. 1). Four of the seven patients who were examined by an ophthalmologist had changes of a cicatrising conjunctivitis

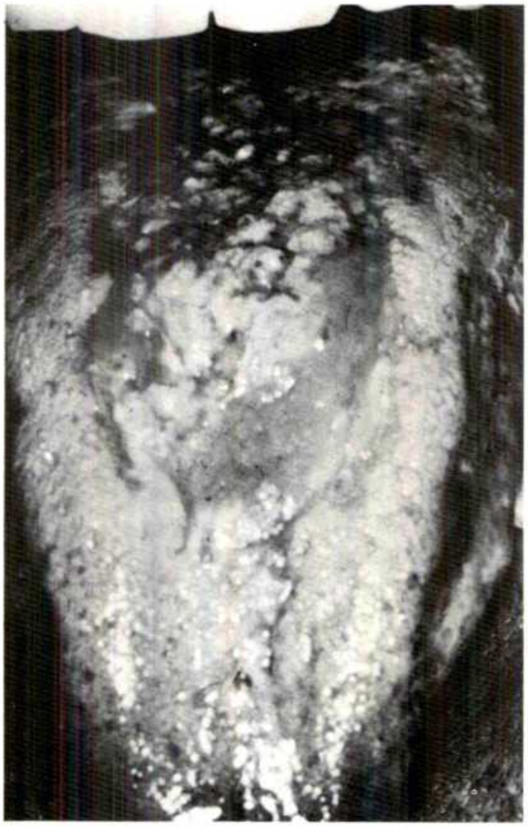

FIGURE 1. Oral ulceration in a patient with linear IgA disease. (Courtesy of Dr E.Rhodes and the Medical Photographic Department, St Hellier Hospital, Surrey.)

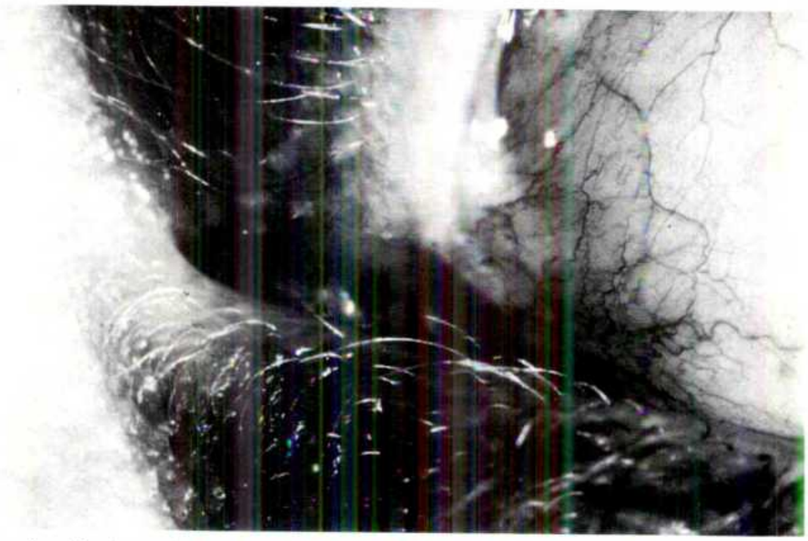

FIGURE 2. Symblepharon formation in a patient with linear IgA disease. This patient had no ocular symptoms.

indistinguishable from those of benign mucous membrane pemphigoid. In two of these patients the ocular involvement was asymptomatic but examination showed loss of inner canthal architecture with shrinkage of the conjunctival sac. In a third patient, who was also asymptomatic, symblepharon formation was present (Fig. 2). In the fourth patient both symptoms and signs of a cicatrising conjunctivitis were present—examination showed cicatricial entropion with trichiasis.

Benign mucous membrane pemphigoid

The clinical features and immunofluorescence findings are summarized in Table 1. Nine patients presented with a history of oral ulceration but no eye symptoms (patients 1–9) and had HL deposits of immunoglobulins in perilesional mucous membrane. Although none of the patients had ocular symptoms, examination of the eyes showed clear changes of a cicatrising conjunctivitis in six patients. It must be noted that four of these six patients had gingival lesions alone (erosive or desquamative gingivitis) (patient nos. 1,2,7,9).

Nine patients (nos. 10–18) presented with ocular symptoms alone, and gave no history of oral blistering or ulceration. All of these patients had signs of a cicatrising conjunctivitis.

Seven patients (nos. 19–25) presented with a history of both oral and ocular symptoms. In four of these patients the oral lesions were in clinical remission at the time of study and immunofluorescence of histologically uninvolved mucosa was negative. In a further three patients (nos. 23–25) active oral lesions were present and immunoglobulins were found in perilesional mucous membrane. The final two patients (nos. 26 and 27) had cutaneous involvement with scarring, in addition to mucous membrane involvement.

Eight of the twenty-seven patients studied had immunoglobulins deposited in an HL pattern in uninvolved skin. IgA was found in three patients. Six of these eight patients had oral ulceration with immunoglobulins in perilesional mucous membrane (patient nos. 4,6,24,25,26,27) and two had eye lesions alone (patient nos. 11 and 17). Apart from the presence of cutaneous lesions in patients 26 and 27, there was no other distinction to be made clinically

TABLE 1. Clinical features and immunofluorescence results in patients with benign mucous membrane pemphigoid

Patient	Sex	Age	Duration of disease (years)	Clinical features: Eye symptoms	Eye signs	Oral lesions	Skin lesions	Immunofluorescence findings: Perilesional oral mucous membrane biopsy	Uninvolved skin biopsy
1. J.E.	M	85	5	−	+	+	−	IgA,IgG,C3	−ve
2. A.G.	M	67	1½	−	+	+	−	IgA	−ve
3. K.S.	F	68	4	−	+	+	−	IgA,IgG,C3	−ve
4. A.M.	M	24	5	−	−	+	−	IgA,IgG,IgM,C3	IgG,C3
5. G.W.	M	69	3½	−	+	+	−	IgA,IgG	−ve
6. I.M.	M	44	8	−	−	+	−	IgG,C3	IgA
7. A.H.	M	61	10	−	+	+	−	IgG,C3	−ve
8. A.C.	M	67	3	−	−	+	−	IgG	−ve
9. D.E.	F	67	½	−	+	+	−	IgG,C3	−ve
10. T.B.	M	81	20	+	+	−	−	No lesion	−ve
11. R.S.	M	73	6	+	+	−	−	No lesion	IgA,IgG,IgM,C3
12. I.W.	F	55	1	+	+	−	−	No lesion	−ve
13. K.C.	F	63	½	+	+	−	−	No lesion	−ve
14. L.G.	F	62	10	+	+	−	−	No lesion	−ve
15. H.D.	F	77	4	+	+	−	−	No lesion	−ve
16. P.S.	M	24	8	+	+	−	−	No lesion	−ve
17. D.G.	F	54	2	+	+	−	−	No lesion	IgG
18. M.V.	F	63	5	+	+	−	−	No lesion	−ve
19. G.H.	M	70	10	+	+	R	−	UI −ve	−ve
20. W.G.	M	58	6	+	+	R	−	UI −ve	−ve
21. G.L.	F	69	4	+	+	R	−	UI −ve	−ve
22. G.B.	M	54	20	+	+	R	−	N.D.	−ve
23. A.C.	M	74	15	+	+	+	−	IgA,IgG	−ve
24. L.G.	M	64	5	+	+	+	−	IgA	IgA
25. E.D.	F	68	3	+	+	+	−	IgA,IgG,C3	IgG,IgM
26. A.M.	F	57	3	+	+	+	+	IgG,IgM,C3	IgG,C3
27. I.F.	F	73	4	+	+	+	+	IgG	IgG,C3

R = in remission at time of study.
UI = histologically uninvolved.

between those patients with, and those without immunoglobulins in uninvolved skin. Furthermore, there was no association between the class of immunoglobulin in either skin or mucous membrane and any specific clinical feature.

AGA were found in six patients (nos 4,6,11,20,21,23) and, in each case, they were of IgG class only. Jejunal biopsies performed in five of these patients showed four to be normal both morphologically and on IELC; the fifth patient (no. 4) had a convoluted mucosa. A further patient (no. 24) with IgA deposited in uninvolved skin, but no AGA in his serum, had a morphologically normal jejunal biopsy but there was a markedly raised lymphocyte count of 652/1000 epithelial cells (upper limit of normal = 250).

ABMZA were found in only two patients in the study. In one patient (no. 24) they were of IgA IgA class and in the other patient (no. 26) of IgG class. In both cases they were of low titre (1:10). Only three patients had HLA-B8 (expected number = 6), and no other A or B locus antigen was found to have an increased incidence.

DISCUSSION

There was a high incidence of mucous membrane lesions in patients with LAD. Nine out of thirty-four had oral ulceration and four out of seven had changes of a cicatrising conjunctivitis indistinguishable from those seen in BMMP. The high incidence of ocular involvement in LAD is important because cicatrisation of the conjunctiva, if progressive, may lead to blindness. Early diagnosis and close follow-up are essential if any attempt is to be made to slow down or arrest the process with topical steroids or immunosuppressive agents. The findings of this study indicate that an ophthalmological assessment is desirable in all patients with LAD regardless of whether they have any ocular symptoms.

A major problem in selecting patients with BMMP for this study was the varied nomenclature that has arisen over the years for describing clinical expressions of the condition. In the oral mucosa, for example, cicatricial pemphigoid, erosive or desquamative gingivitis and non-dystrophic epidermolysis bullosa are all clinical variants of the disease. For the purposes of this study, the patients were selected either by having oral blistering and ulceration, compatible histologically with BMMP and with HL deposits of immunoglobulins in perilesional mucosa, or by having symptoms and signs of a cicatrising conjunctivitis for which other causes had been excluded on clinical criteria. Although the Stevens–Johnson syndrome, chemical burns and trachoma virus can cause a cicatricial conjunctivitis, these conditions can be distinguished on clinical grounds. Previous studies have been performed in which immunofluorescence on conjunctival biopsies showed HL deposits of immunoglobulins (Furney *et al.*, 1975). The conjunctiva was not biopsied in this study because it was felt that there was a significant risk of accelerating the scarring process.

Of the nine patients who presented with oral ulceration alone, six had signs of a cicatrising conjunctivitis despite the absence of ocular symptoms. Four of these patients had the clinical picture referred to as erosive or desquamative gingivitis (Fine & Weathers, 1980), or non-dystrophic epidermolysis bullosa (Forman & Nally, 1977), which was considered to be a *forme fruste* of BMMP. The finding in this study, that these patients have a cicatrising conjunctivitis, supports the view that they should be considered as having BMMP. It is not known whether all patients presenting with oral lesions alone will develop a cicatrising conjunctivitis, nor is it known whether presymptomatic treatment will help to slow down or arrest the conjunctivitis. Further information on these points will only become known by careful long-term follow-up of these patients.

Perilesional biopsies of oral mucous membrane showed a variety of immunoglobulin classes to be deposited, but there was no correlation of the class with the clinical features. The finding that eight patients with BMMP had immunoglobulins in uninvolved skin is hard to evaluate, especially in the six patients with no history of skin blisters. In the other two patients, however, cutaneous lesions were present suggesting that the immunoglobulin deposits were part of the clinical pattern. There was no constant relationship between the class of immunoglobulin in the skin and the clinical presentation of the patient. Of particular interest were the three patients with BMMP who had HL deposits of IgA in uninvolved skin (patient nos. 6,11,24). In view of the high incidence of mucous membrane lesions in patients with LAD it may be that LAD presents as a clinical spectrum ranging from patients with only mucous membrane lesions to those with only skin involvement.

AGA were found in six of the twenty-seven patients with BMMP. In all cases they were of IgG class and only one patient out of five biopsied had an abnormal jejunal mucosa. In a previous study of patients with dermatitis herpetiformis, it was found that AGA were a good screening test for gluten-sensitive enteropathy (Unsworth *et al.*, 1981). In most cases, however, the correlation was best with AGA of IgA class as is the case with coeliac disease (Unsworth, Walker Smith & Holborow, 1983). In a recent study of patients with bullous pemphigoid, Kieffer & Barnetson (1981) found significantly raised levels of AGA of both IgA and IgG class, but none of their patients had a jejunal biopsy. However, Marks & Shuster (1969) reported a series of ten patients with bullous pemphigoid all of whom had normal jejunal biopsies on dissecting microscope appearance. Clearly, our eventual explanation of these diseases must account for these anomalous findings. One additional patient, who had no AGA in the serum, but who had an HL deposit of AGA in uninvolved skin, had a jejunal biopsy. This showed a markedly elevated IELC, in keeping with our previous report that a detectable enteropathy is found in 24% of patients with LAD (Leonard *et al.*, 1982).

The low incidence of ABMZA is consistent with previous reports in BMMP (Bean *et al.*, 1979), and is similar to the low incidence in LAD. There was no increase in the incidence of HLA-B8 in patients with BMMP, unlike the findings in dermatitis herpetiformis and LAD where the incidence is 88% and 56% respectively (Leonard *et al.*, 1982). No other A or B locus antigen was found in raised incidence in contrast to the report of Mondino and colleagues (1979) who found a raised incidence of HLA-B12.

In conclusion, this study has shown that patients with LAD and BMMP should be examined by an ophthalmologist for signs of a cicatrising conjunctivitis, even if there are no ocular symptoms. The laboratory findings are conflicting and less straightforward to interpret. The study has shown that the application of currently available routine techniques does not allow for satisfactory classification of the bullous dermatoses. Although immunofluorescence is the best technique yet available for diagnosing and classifying them, further understanding of these diseases must await the time when antigenic determinants can be recognized and isolated for characterization.

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Evidence that the IgA in patients with linear IgA disease is qualitatively different from that of patients with dermatitis herpetiformis

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SUMMARY

A previous study using immunofluorescent techniques showed J-chain to be present in the uninvolved skin of patients with papillary IgA dermatitis herpetiformis (DH) in a distribution that was coextensive with the IgA. This implied that the IgA was dimeric and of mucosal origin. In this study, fifteen patients with papillary IgA deposits, fifteen with homogeneous-linear (HL) IgA deposits and four patients with granular-linear (GL) IgA deposits were tested for the presence of *in vivo* bound J-chain.

All fifteen patients with papillary IgA deposits and all four with GL IgA deposits had J-chain staining coextensive with the IgA. However, only one of fifteen patients with HL IgA deposits demonstrated *in vivo* bound J-chain that could not be accounted for by coexisting IgM deposits. These findings indicate that the IgA in patients with HL deposits is qualitatively different from that in patients with papillary and GL IgA deposits and makes the distinction between the two types of linear fluorescence particularly important.

Patients with dermatitis herpetiformis have deposits of IgA in the dermal papillae of uninvolved skin. The disease is associated with gluten-sensitive enteropathy (GSE) (Fry *et al.*, 1967; Shuster, Watson & Marks, 1968). Both the rash and the GSE respond to treatment with a strict gluten-free diet (Fry *et al.*, 1968; Reunala *et al.*, 1977), and relapse on gluten challenge (Leonard *et al.*, 1983). The association between the skin and the gut is supported by the finding that over 80% of patients with DH have HLA-B8, a similar incidence to that in coeliac disease (Seah *et al.*, 1976). This association has been further strengthened by the recent finding that the IgA deposits in the skin of patients with DH contain J-chain and are able, *in vitro*, to bind secretory component (SC). This implied that the IgA was dimeric and originated from a mucosal surface—most probably the gut (Unsworth *et al.*, 1982).

A rarer bullous disease has been recognized in which the IgA was deposited in a linear fashion along the dermo-epidermal junction (Chorzelski *et al.*, 1971; Fry & Seah, 1974). Two patterns of linear deposition have recently been described (Leonard *et al.*, 1982): homogeneous-linear (HL) and granular-linear (GL). In patients with the HL pattern, the deposit is seen as a smooth unbroken line, whilst in the GL pattern, a broken line is seen along the dermo-epidermal junction. There has been much controversy over recent years as to whether the linear patterns should be considered as a separate entity from DH, or whether they are a subgroup of DH with the inherent implication of gluten sensitivity (Honeyman *et al.*, 1973; Chorzelski *et al.*, 1979; Russell Jones & Goolamali, 1980). There is a much lower incidence of enteropathy and of HLA-B8 in the linear groups than in DH (Lawley *et al.*, 1980; Leonard *et al.*, 1982).

This study was designed to investigate the presence of J-chain in the skin of patients with HL and GL IgA deposits and to relate it to the presence of coexisting enteropathy and HLA-B8. The study would thus provide useful information as to the origin of the IgA and hence to the pathogenesis and nosology of these diseases.

METHODS

Four-mm punch biopsies were taken under local anaesthesia from the uninvolved skin of fifteen patients with papillary IgA, fifteen with HL IgA and four with GL IgA deposits. One normal subject and one patient with pemphigus were used as controls. All patients studied were over fifteen at the onset of their disease and all were eating a normal diet at the time of study. The skin biopsies were embedded in OCT medium (Lab-Tek Inc.) and snap-frozen in liquid nitrogen. They were stored in liquid nitrogen until processing.

The reagents used were as follows: FITC sheep anti-human IgA, IgG, IgM and C3, and FITC swine anti-rabbit immunoglobulin (Dako Reagents) with a fluorescein:protein ratio of 2·3; TRITC goat anti-human IgA (Nordic Reagents) with a rhodamine:protein ratio of 3·0; rabbit anti-human J-chain (Nordic Reagents). The specificities of the conjugated reagents were tested by double diffusion against the antigen. The specificity of the anti-human J-chain was determined by double diffusion and immunoelectrophoresis against a J-chain reference serum (Nordic Reagents).

Five-micron cryostat sections of the skin biopsies were tested for the presence of *in vivo* bound immunoglobulins and C3 by direct immunofluorescence. Further 5-μm cryostat sections were trypsinized by incubation with 0·1% trypsin (Difco Ltd.) in aqueous calcium chloride at 37°C for 30 min. The sections were then washed in phosphate-buffered saline and double stained for the presence of IgA (with TRITC goat anti-human IgA), and for J-chain (by indirect immunofluorescence using the rabbit anti-J-chain and the FITC swine anti-rabbit immunoglobulin). In all cases controls were run by substituting normal sera of the same species for the specific antibody.

Fluorescent microscopy was performed using an Olympus BH 313 microscope with fluorescent vertical illumination from an HBO 100 Watt mercury arc, a dichroic mirror 20 BC-W/EY 455 and a barrier filter from 0 to 530 nm.

Jejunal biopsies were performed from all fifteen patients with papillary IgA deposits, thirteen of the fifteen with HL IgA deposits and all four with GL IgA deposits, using a Crosby capsule. The biopsy was assessed for morphological change using a dissecting microscope, and an intra-epithelial lymphocyte count (IELC) was performed on H & E sections of the biopsy as described previously (Fry *et al.*, 1974). The IELC was expressed as lymphocytes per 1000 epithelial cells—the upper limit of normal was taken as 250. Venous blood was taken from

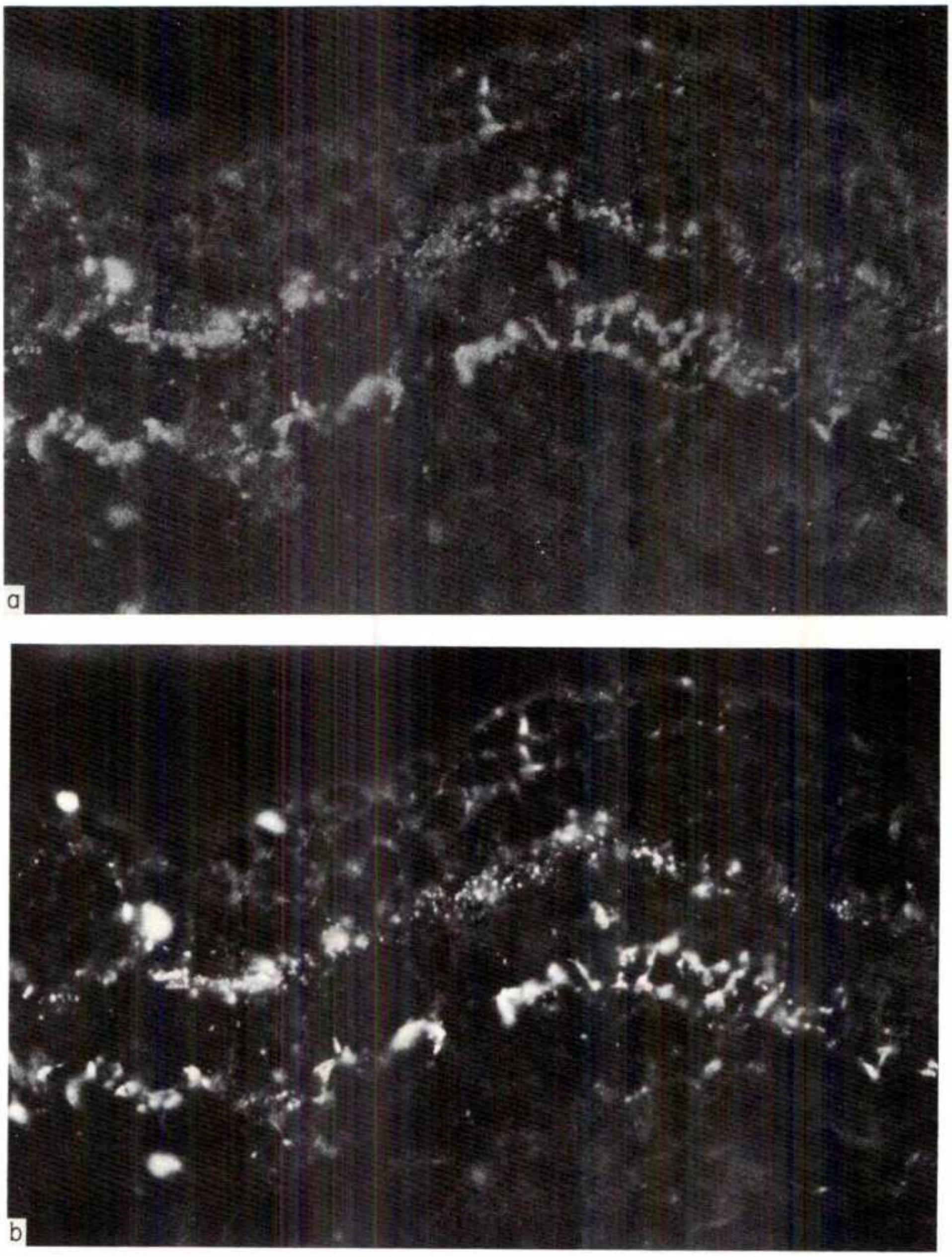

FIGURE 1. (a) Papillary IgA staining demonstrated with TRITC anti-human IgA on a trypsinized section of skin from a patient with DH. (b) The same section stained with FITC anti-human J-chain, showing the J-chain staining to be coextensive with the IgA.

fourteen of the HL and all four of the GL patients for HLA typing of A and B loci using the NIH microcytotoxicity test (Terasaki & McClelland, 1964).

RESULTS

Patients with papillary IgA deposits

Four of the fifteen patients studied also had papillary IgM deposits, three had papillary IgG deposits and three had papillary C_3 deposits. *In vivo* bound J-chain was demonstrated in all fifteen patients in a pattern that, on double staining, was coextensive with the IgA (Fig. 1). Twelve of the fifteen patients had an abnormal jejunal biopsy.

Patients with linear IgA deposits

The results are summarized in Table 1. IgM was demonstrated in an HL pattern in four patients with HL deposits but none with GL deposits of IgA. All four of the patients with HL IgM had *in vivo* bound J-chain demonstrated as did one other patient (no. 6) with no IgM. None of the other HL IgA patients had *in vivo* bound J-chain. All four patients with the GL pattern of IgA deposition were positive for J-chain.

Two of the twelve patients in the HL group who had jejunal biopsies performed had a macroscopic abnormality (patient nos. 1 and 12) a further two patients had a raised IELC

TABLE 1. Summary of findings in patients with linear IgA deposits

			Immunofluorescence findings					Small intestinal biopsy		
Patient	Sex	Age	IgA	IgG	IgM	C_3	J-chain	Morphological	IELC	Presence of HLA-B8
1	F	82	HL +	–	–	–	–	Convoluted	198	+
2	M	42	HL +	–	HL +	HL +	HL +	Normal	156	–
3	F	31	HL +	–	–	–	–	Normal	121	+
4	M	60	HL +	–	–	–	–	Normal	323	+
5	F	58	HL +	–	–	HL +	–	Normal	128	–
6	M	21	HL +	–	–	GL +	HL +	Normal	192	+
7	M	75	HL +	–	–	–	–	ND	ND	+
8	M	78	HL +	–	–	–	–	Normal	213	+
9	F	39	HL +	–	HL +	GL +	HL +	Normal	154	–
10	M	53	HL +	HL +	–	–	–	ND	ND	–
11	F	23	HL +	–	–	–	–	Normal	334	+
12	F	69	HL +	–	–	HL +	–	Convoluted	512	ND
13	M	22	HL +	HL +	HL +	–	HL +	Normal	102	+
14	M	65	HL +	–	–	–	–	Normal	144	+
15	F	42	HL +	–	HL +	–	HL +	ND	ND	+
16	F	51	GL +	–	–	–	GL +	Normal	211	–
17	F	76	GL +	HL +	–	GL +	GL +	Normal	221	+
18	M	45	GL +	–		P +	GL +	Normal	201	–
19	M	46	GL +	–	–	P +	GL +	Normal	334	+

IELC = Intra-epithelial lymphocyte count (expressed as number of lymphocytes per 1000 epithelial cells).
ND = not done.

(patient nos. 4 and 11). Only one of the four patients with GL IgA deposits had an abnormal small intestinal biopsy (patient no. 19) and this was only at histological level. Ten of the fourteen patients with HL deposits who were tissue typed had HLA-B8 as did two of the four with GL IgA deposits.

Controls

The normal skin control showed no fluorescence with antisera against IgA, IgG, IgM, C3 or J-chain. The skin from the patient with pemphigus showed intercellular staining with the anti-human IgG but was negative to all other reagents tested. No fluorescence was observed when the specific antisera were substituted by normal sera from the same species.

DISCUSSION

The early studies with immunofluorescence took patients with linear IgA deposits to be a variant of DH comprising up to 14% of this group (Chorzelski *et al.*, 1971; Fry & Seah, 1974). This classification has an important implication as DH is now generally regarded as being caused by gluten sensitivity (Fry *et al.*, 1967; Reunala *et al.*, 1977). Chorzelski and his colleagues (1979) were the first to suggest that patients with linear deposits of IgA constituted a distinct entity to be differentiated from both DH and pemphigoid, and they proposed the term linear IgA bullous dermatosis. Lawley and his colleagues (1980), in a study of ten patients with linear IgA deposits, considered that these individuals had a disease that was pathophysiologically different from DH. Of particular relevance were the absence of a detectable enteropathy in the six patients who had a small intestinal biopsy, and the lower incidence of HLA-B8 (30% as opposed to 80% in DH). However, in a recent multi-centre study of forty-two patients with linear IgA depostis the situation was not clear cut (Leonard *et al.*, 1982). Firstly, two patterns of linear fluorescence could be distinguished: the HL pattern found in thirty-four patients and the GL pattern found in eight patients. In the HL group, 24% had a demonstrable enteropathy and 56% HLA-B8, suggesting that there were two subgroups of patients with HL deposits—one with the same pathogenesis as DH and a second group with a different pathogenesis. The GL group had an incidence of enteropathy of 36% and of HLA-B8 50%. However, only eight patients were studied and this was considered to be too small a group for worthwhile comment on its relationship to DH. The term linear IgA disease was reserved for patients with HL IgA deposits.

J-chain is a protein produced by plasma cells. In IgA producing plasma cells of mucosal surfaces the J-chain links two IgA monomers of the same antigenic specificity to form an IgA dimer. Dimeric IgA can be distinguished from monomeric IgA by the presence of J-chain. The production of dimeric IgA in mucosal sites is important because only IgA dimer is able to bind with SC. The IgA dimer–SC complex is then transported across the epithelial cells to the mucosal surface. Although the precise details of IgA metabolism in man have not yet been established, it is known that over 90% of serum IgA circulates in a monomeric form. This suggests that non-mucosal sites produce monomeric and not dimeric IgA.

Expression of J-chain is not the sole prerogative of the dimeric IgA molecule. IgM circulates as a pentamer containing J-chain. Therefore it was expected that J-chain would be demonstrated in both *in vivo* bound IgM and IgA dimer deposits. Trypsinization of the sections was required to enhance the J-chain staining. This is presumably because trypsin opens up the immunoglobulin–J-chain complex and makes the cryptic antigenic components available to the J-chain antiserum.

Only four of the fifteen patients with papillary IgA deposits had coexisting IgM, but all fifteen were J-chain positive. In the GL group none had IgM deposits but all four demonstrated *in vivo* bound J-chain. In the HL group four patients had coexisting IgM; these four patients and one other were positive for J-chain staining. The remaining ten patients were J-chain negative irrespective of the presence of a coexisting enteropathy or HLA-B8.

It is not clear why one of the patients with HL IgA deposits demonstrated positive J-chain staining in the absence of IgM. It could be that in this individual the clones of plasma cells producing the IgA were of mucosal origin. An alternative possibility was that free J-chain was present in the skin, though this seems less likely.

In general, the results of this study suggest that patients with HL deposits of IgA represent a different disease entity from patients with papillary IgA deposits. The IgA in the GL group, however, appears to be dimeric, indicating that this disease should be regarded as synonymous with DH. This finding makes the distinction of the GL and HL patterns of immunofluorescence particularly important.

In conclusion, this study provides further evidence that linear IgA disease and DH have a different pathophysiology. However, there remain many similarities between the two disorders, one of which is that they both have IgA deposited in uninvolved skin. The situation may be resolved with more certainty when satisfactory methods for eluting the IgA from the skin are developed which will render it suitable for detailed analysis.

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The patients with linear IgA deposits described in this study formed part of a multi-centre study reported previously. We would again like to express our thanks to all those physicians who participated.

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Cutaneous anaesthesia in necrobiosis lipoidica

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SUMMARY

Cutaneous sensation in plaques of necrobiosis lipoidica was assessed in five non-diabetic and seven diabetic patients. Eleven of the twelve showed partial or complete anaesthesia of the affected skin. These findings are important in the differential diagnosis of tuberculoid leprosy. Further studies of nerve function in cutaneous granulomas need to be carried out.

A 38-year-old English woman who had spent a number of years working in Thailand presented in our Department with a diagnosis of a single plaque of tuberculoid leprosy on the shin. The patient had complained, and continued to complain, that it was anaesthetic. The diagnosis had been made in Bangkok and accompanying the patient there was a pathologist's report on a biopsy of the lesion which confirmed the diagnosis including nerve damage. The patient was receiving dapsone.

In our Department anaesthesia to light touch, heat, cold, and pin-prick of the lesion which measured 3·0 × 3·0 cm was confirmed on many occasions. The plaque was smooth, hypopigmented and dry. There was no palpable nerve enlargement.

The original histology was reviewed by a leprologist (Dr A.C.McDougall, The Slade Hospital, Oxford) and the nerve bundle structure was normal; no definite evidence of tuberculoid leprosy was found. A further biopsy was performed which showed the characteristics of necrobiosis lipoidica and no nerve abnormality. Extensive destruction of collagen was demonstrated by the Alcian Blue stain and *Mycobacterium leprae* were not found on Wade-Fite and auramine–rhodamine stained sections.

Dapsone was discontinued, no evidence of diabetes mellitus was found and the patient was delighted at the changed diagnosis.

As we were unable to find any reports of sensory change in plaques of necrobiosis lipoidica, we decided to study this in other patients, both with and without diabetes.

METHODS

Twelve patients with necrobiosis lipoidica affecting one or more limbs were assessed and four of these had a previous skin biopsy confirming the diagnosis. Seven patients were diabetic and five were not. The plaques were tested by one of us for light touch with cotton wool, heat and cold with tubes of water, and pin-prick in the normal way for neurological assessment.

The response was graded as being normal, decreased or absent. Similar testing of clinically normal skin was carried out at the same time in order to detect the possible presence of peripheral neuropathy.

RESULTS

Of the twelve patients eleven were female. Five gave a past history of ulceration of the necrobiosis lipoidica and in three it was still present. The mean age of the patients was 42 years and the mean duration of the plaques 9 years. Ten had lesions on the shins and/or ankles, one had lesions on the arms and legs, and one a lesion on the forearm. Of the twelve patients, five showed complete absence of sensation to light touch and five to hot and cold. Four showed complete absence to pin-prick.

Nine showed a complete absence of at least one modality and eleven a decrease or absence of at least one cutaneous sensory modality (Table 1).

TABLE 1. Sensory findings in necrobiosis lipoidica

No.	Age of patient (years)	Duration of necrobiosis (years)	History of ulceration	Light touch sensation	Pin-prick sensation	Heat/cold sensation
1	80	6	Present	N	N	↓
2	54	12	None	N	N	N
3	56	32	None	A	A	↓
4	37	5	None	A	↓	↓
5	72	20	Past	↓	↓	A
6	23	5	Past	↓	↓	N
7	32	10	Past	↓	N	A
8	19	4	Past	A	A	A
9	50	< 1	Present	↓	↓	A
10	28	4	Past	↓	A	↓
11	28	7	Present	A	↓	↓
12	25	2	None	A	A	A

Sensation normal (N), decreased (↓), absent (A).
Patients 6 to 12 were diabetic.
Patient 11 had peripheral neuropathy of the feet.

Eight showed a diminution in all three sensory modalities. Of the four patients who had no previous history of ulceration of lesions, three showed sensory impairment.

There was no clear difference in the findings between those with and without diabetes. Three patients complained of itching mainly at the edges of the plaques. Peripheral neuropathy was found involving the feet of one diabetic, but this did not extend up to the necrobiotic plaque of the leg.

DISCUSSION

Demonstration of the loss of sensation, especially light touch and temperature, is one of the prime diagnostic criteria in the diagnosis of tuberculoid leprosy. Occasionally the patient may actually present with the complaint that the skin lesion is anaesthetic. Our findings show the futility of relying on such findings where the true diagnosis is necrobiosis lipoidica. Sensory change in other types of cutaneous granulomata is little considered by physicians and is rarely tested for unless tuberculoid leprosy enters into the differential diagnosis.

Plaques of necrobiosis lipoidica are usually asymptomatic though burning and irritation/itching may occur (Hildebrand *et al.*, 1940). Partial or complete anaesthesia has not been previously reported, though it was present in all except one of our patients. Perhaps occult nerve damage is occurring in the necrobiotic process and the irritation noted at the edge of the plaques by three of our patients may be due to neural entrapment in the necrobiotic process. Sensory impairment as an indicator of tuberculoid leprosy may, therefore, be misleading and we suggest that there is a need for further study of sensation in other chronic inflammatory and granulomatous cutaneous disorders. Important examples are granuloma annulare, lupus vulgaris, sarcoidosis, chronic leishmaniasis and granuloma multiforme.

The mechanisms of cutaneous sensation have been subjected to much recent study and the old simplistic ideas of limited modalities which fail to account for such sensations as stickiness, hardness, roughness, greasiness and clamminess are being replaced by the concept of preferential receptivity. This concept assumes the plasticity of central nerve pathways that have preferential though not exclusive functions (Sinclair, 1981).

Hatzis *et al.* (1983) have studied sweat gland disturbances in necrobiosis lipoidica and granuloma annulare. Using a combination of the plastic impression and starch iodine methods they have demonstrated an intense and uniform hypohidrosis in necrobiosis lipoidica, whereas in granuloma annulare they found anhidrosis or hypohidrosis far more intense at the active periphery of the lesion. Our original patient's lesion was smooth and dry as is also found in tuberculoid leprosy. Eccrine sweat gland function though under central control is dependent upon intact local innervation. Their findings may well provide further evidence of pathological impairment of nerve function in necrobiosis lipoidica as well as adding difficulty to the differential diagnosis of tuberculoid leprosy.

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Studies on the mechanism of clinical tolerance in solar urticaria

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SUMMARY

Tolerance to artificial ultraviolet radiation (UVR) was induced in three patients with solar urticaria by administering graded whole body exposures to long-wave ultraviolet radiation (UV-A, 320–400 nm) in a phototherapy cabinet. Plasma histamine levels, mast cell ultrastructure and cutaneous responses to intradermally injected codeine and histamine were examined before and after the induction of tolerance. No evidence of serum complement activation could be demonstrated following exposure of serum samples to UVR *in vitro*. These studies suggest that the state of tolerance is due neither to mediator (histamine) depletion nor to a systemic effect induced by UV-A but may be due to an increase in the mast cell degranulation threshold.

Patients with idiopathic solar urticaria usually develop a brisk weal and flare reaction within minutes of exposure to sunlight. The urticarial response is transient, disappearing within 30 to 60 minutes if further exposure to sunlight is avoided. The condition, however, can be potentially serious. Exposure of large areas of skin can result in extensive urticaria and hypotension leading to loss of consciousness or shock. Drug therapy for solar urticaria has been disappointing. H_1 and H_2 antihistamines, singly or in combination, are only marginally effective and generally do not provide satisfactory clinical relief (Michell *et al.*, 1980).

Repeated irradiation of the skin at short intervals may result in a progressively diminishing cutaneous response and it is this phenomenon that forms the basis for the induction of clinical tolerance by graded exposure to ultraviolet or visible radiation (Ramsay, 1977). The induction of tolerance is the single most effective treatment for solar urticaria, yet the mechanism for this state of refractoriness has not been clarified.

In the present investigation, the mechanism by which repeated exposures to artificial ultraviolet radiation leads to tolerance was examined. Plasma histamine levels, mast cell ultrastructure, and cutaneous responses to intradermal codeine and histamine were measured

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before and after induction of tolerance in patients with solar urticaria. An attempt was also made to determine if complement activation occurred in response to UVR.

METHODS

The details of the patient's clinical findings are given in Table 1. Written informed consent was obtained. No patient had been taking antihistamines or other drugs for the previous 7 days. All patients developed urticaria in less than 15 minutes after sun exposure, the reaction usually subsiding within 30 minutes following protection from further exposure. Solar urticaria was not associated with any other photodermatosis and had developed spontaneously with no apparent precipitating cause. In all cases, red blood cell and plasma protoporphyrin levels were within normal limits.

Light sources and radiometry

A compact 150 Watt Xenon arc solar simulator was used for the experimental induction of urticaria. Briefly, the collimated beam was directed onto a UVR reflecting dichroic mirror at 45° incidence. The reflected radiation was then passed through a short wavelength cut-off filter (Schott WG320, 2 mm thickness) to produce 'solar simulated' radiation (290–400 nm). A broad UV-A waveband (320–400 nm) was obtained from the same source by additional filtration through a Schott WG 345 filter (1·5 mm thickness, approximate 50% cut-off at 341 nm) and through a coloured UG 11 (1 mm) filter to attenuate remaining visible wavelengths. The resulting continuous spectrum extended from 320 to 410 nm, with a peak at about 350 nm. A broad UV-B waveband (290–320 nm) was also obtained from the same source by passing the reflected beam from the dichroic mirror through (i) a short wavelength cut-off filter (Schott WG305, 2 mm thickness, 50% cut-off at about 300 nm), (ii) a Schott UG 11 filter (1 mm) and (iii) a narrow band interference filter (Infrared Industries) with peak transmission at 300 nm and half-power bandwidth of about 12 nm. The final spectrum was roughly a bell-shaped curve extending from 285 to 330 nm, with a peak at 300 nm. Visible wavelengths (400–800 nm) were obtained by passing the reflected beam through a 3 mm Schott GG 495 filter (50% cut-off at approximately 495 nm). An International Light Spectroradiometer system (model 782) was used to determine the spectral distribution at the exit. The irradiated skin area was a 1·0 cm circle. Intensity was measured by a calibrated thermopile (Eppley Laboratories) attached to a Keithly millimicrovoltmeter (model 149). Average irradiance at skin level was 76·5 mW/cm^2 for solar-simulated radiation; 0·70 mW/cm^2 for UV-B and 29·1 mW/cm^2 for UV-A.

An unfiltered cold quartz Fischer lamp (low pressure mercury vapour) was used as a source of short ultraviolet wavelengths. Over 90% of the emission of this source is around 254 nm. Irradiance was also measured by a calibrated thermopile.

UV-A irradiance in a phototherapy cabinet and in a circular reflector equipped with seventeen 20 W fluorescent blacklight tubes for the irradiation of the forearm (see below) was measured by a calibrated photodiode-type cosine-corrected detector (SEE-010) with response range from 300 to 400 nm (peak response at 365 nm) attached to an IL700 research radiometer (International Light, Inc.).

Phototesting

The minimal wealing dose (MWD) with each of the wavebands mentioned above was determined over the mid or lower back in each patient before and after the induction of clinical tolerance. A protection factor was determined in each instance as follows:

$$\frac{\text{Minimal wealing dose (post-tolerance)}}{\text{Minimal wealing dose (pre-tolerance)}}$$

The minimal erythema dose (MED) using solar simulated radiation was also determined over the lower back prior to and following the induction of clinical tolerance by giving a series of exposures in 25% increments. The MED was recorded 20–24 h later.

Induction of tolerance

Clinical tolerance to UVR was induced by repeated exposure to UV-A in a National Biologic PUVA unit equipped with forty-eight General Electric blacklight fluorescent tubes. The emission spectrum of the tubes extended from 310 to 400 nm with a peak at 360 nm and the average UV-A irradiance in the centre of the unit was 4·2 mW/cm^2. Initially, only the face, forearms and legs were exposed every 60 min to doses sufficient to provoke immediate erythema or wealing (0·5–1·0 J/cm^2) until the sites become refractory. Additional sites were then exposed (thigh, arms, trunk) to similar doses until the entire body surface became unresponsive. The same dose was given repeatedly at 60-min intervals until the exposed skin became unresponsive before raising the dose by approximately 1 J/cm^2. An average of three exposures to the same dose level were required before the skin became tolerant.

Pharmacological studies

After collection of a baseline blood sample, erythema and wealing were evoked by exposure to UV-A from a circular reflector equipped with seventeen Westinghouse 20 blacklight fluorescent tubes placed symmetrically about the hand and forearm. Patient 1 received a total dose of 0·9 J/cm^2, while patients 2 and 3 received 0·8 J/cm^2. Three ml blood samples were taken from an indwelling antecubital vein catheter prior to irradiation and at intervals up to 30 min post-irradiation and admixed with 100 units of heparin. Venous blood samples were also taken periodically from the contralateral (non-irradiated) forearm of patients 2 and 3. Following the induction of clinical tolerance the patients' forearms were similarly irradiated and blood samples again collected at similar intervals. All samples were centrifuged at 4°C for 15 min at 1500 r.p.m. Plasma was then separated and stored at −20°C.

Samples of plasma were assayed in triplicate for histamine by means of the microenzymatic method of Levy and Widra (Levy & Widra, 1973). This technique reproducibly detects elevations in histamine levels above 5 ng/ml in plasma, whereas differences between levels below 5 ng/ml are not significant (Atkins, Valenzano & Zweiman, 1982).

Activation of complement

Undiluted serum from patients 2 and 3 and from two normal healthy volunteers was irradiated *in vitro* for 15 min (about 70 J/cm^2) with solar-simulating radiation. Residual haemolytic activity was assayed against antibody-coated sheep erythrocytes by the method of Mayer (Mayer, 1961). Immunoelectrophoresis of serum samples was performed in 1% agarose (Seakem, Rockland, Me.) in high resolution buffer (Gelman, Ann Arbor, Mich.). Slides were developed with rabbit antiserum to human C3 and factor B. Similar immunoelectrophoretic analysis of serum samples from venous blood draining the irradiated 'wealing' forearm of patient 1 was also performed.

Intradermal histamine and codeine injections

Different concentrations of codeine [100 μg/0·1 ml (3 mM) and, 25 μg/0·1 ml (0·8 mM)] and histamine [2 μg/0·1 ml (0·18 mM) and, 0·5 μg/0·1 ml (0·045 mM)] were prepared in normal physiological phosphate-buffered saline and injected intradermally into the upper back before and after induction of tolerance. The resulting weal and flare was traced 15 min post-injection and the area under the tracing was calculated using a planimeter.

Light and electron microscopy

Following peripheral four quadrant injections of 1% xylocaine, 4-mm punch biopsies were taken from the forearm skin of each patient (i) prior to irradiation, (ii) 15 min after UVA-induced wealing and (iii) 15 min after a similar exposure to UV-A following the induction of tolerance.

One portion of each biopsy was placed in formalin and processed for routine paraffin histology. The other portion was fixed and processed for transmission electron microscopy as previously discussed (Lavker, 1979). All sections were coded and evaluated. Evaluations of mast cell alterations were based on inspection of photographs of four adjacent sections in each of two grids from each tissue block. At least three blocks of tissue were sampled in each biopsy specimen. In this way, a minimum of thirty mast cells were inspected. Examination of multiple tissue sections was also used to help evaluate the topographic relationship between altered granules and the mast cell membrane.

Several patterns or degrees of mast cell changes were seen by electron microscopy. These were graded on a 0 to 3 semiquantitative scale. A grade of 0 refers to oblong or oval-shaped cells with numerous microvilli and a cytoplasm filled with electron-dense granules exhibiting a variety of structural configurations including 'whorls and scrolls' and a lattice pattern. A grade of 1 is assigned to cells where the minority (generally about 20%) of granules within each cell are grossly altered with swelling, decreased electron density, and/or decreased structural organization. In some altered granules, a distinct electron-lucid zone was present just below the membrane. The cell retains the oblong or oval configuration. Altered granules are frequently flocculent and fused into masses. Grade 2 refers to cells whose outline is rounded with few microvilli. At least half of cytoplasmic granules are swollen and flocculent. The perigranular membranes of adjacent granules are fused to each other and/or the cell plasma membrane, with evidence of exocytosis of granules into the extracellular milieu. Grade 3 cells are swollen, flocculent, and fused into masses. Altered granules are often found encased by trabeculae of cytoplasm. Exocytosis of granules is common with granule remnants often noted free in the dermis.

Four-mm punch biopsies were also obtained from patient 1 from (i) the normal forearm skin, (ii) a weal provoked by exposure of the anterior forearm skin to UV-A (1·7 J/cm^2) and (iii) a similar site exposed to the same UV-A dose following induction of tolerance. These specimens were processed by direct immunofluorescence for IgG, IgM, IgA, C3 and fibrinogen.

RESULTS

Phototesting

The minimal wealing dose (MWD) with the various broad wavebands in each patient is shown in Table 1. All three patients were sensitive to UV-A and the MWD with this waveband ranged from 0·06 J/cm^2 to 0·8 J/cm^2. Following the induction of clinical tolerance, the patients' tolerant skin over the lower back and non-tolerant (UVA-protected) buttock skin was similarly irradiated. There was no change in the MWD in the protected buttock skin. No clinical reaction could be evoked in the tolerant skin of the lower back despite exposure to doses which were 37 to 60 times that of the pretreatment MWD. This represented a protection factor of more than 60

TABLE 1. Clinical details of patients with solar urticaria

Patient	Age (years)	Sex	Duration of symptoms (years)	Range of spectral sensitivity (nm)	Minimal wealing dose (J/cm²)
1	20	M	1·5	254*	$3·9 \times 10^{-2}$
				290–320	$7·4 \times 10^{-3}$
				320–400	$14·4 \times 10^{-2}$
2	59	M	10	290–320	$8·2 \times 10^{-3}$
				320–400	$5·7 \times 10^{-2}$
3	32	F	3	320–400	0·8

*Unfiltered cold quartz (germicidal) lamp.

for patients 1 and 2 and more than 37 for patient 3. There was no alteration in the MED to solar-simulated radiation following the induction of clinical tolerance.

Induction of clinical tolerance

The number of exposures and total dose given for the induction of tolerance ranged from 19 to 25 (mean = 23) and 12·5–20·2 J/cm² (mean = 15·9) respectively. All patients were hospitalized during the treatment period to avoid exposure to sunlight. Erythema, pruritus and weals generally developed following the first 2–3 exposures. As the skin became less responsive, only erythema appeared after irradiation without wealing or pruritus. Patient 2 experienced a hypotensive episode during the early treatment period and developed oedema and a few petechiae over the lower extremities.

After the induction of tolerance, an unresponsive area over the arm was shielded from light. Small areas (1 × 1 cm) within the shielded tolerant skin were subsequently exposed to UV-A (1 MWD) every 24 h to determine how long tolerance persisted without further treatment. All patients began to develop reactions (weals and/or erythema) by 48 h.

TABLE 2. Plasma histamine levels (ng/ml) in venous blood following challenge with UV-A before and after induction of tolerance

Patient		Sampling time (minutes after UV-A exposure) 0	2	6	10	12	15	20	30
1	Pre-tolerance	3·9	2·9	2·9	5·4	6·5	7·0	5·4	4·9
	Post-tolerance	0	2·0	1·6	1·6	2·4	0	2·4	ND†
2	Pre-tolerance	4·3	2·9	11·8	10·6	7·3	6·5	5·2	2·4
				2·1*		2·0*			
	Post-tolerance	4·4	2·3	4·8	3·5	3·6	6·5	5·1	2·7
3	Pre-tolerance	4·3	5·4	6·0	5·9	6·1	3·7	4·7	3·7
				4·1*	4·3*				
	Post-tolerance	3·2	3·8	3·9	3·4	3·4	2·8	ND	ND

*Plasma samples draining contralateral non-irradiated forearm prior to tolerance.

†ND = not done.

Maintenance doses of UV-A equal to the last dose given for induction of tolerance were given once every 24 h 4–5 days before the patients were discharged. Patient 2 occasionally experienced sparse asymptomatic micropapular wealing during maintenance which resolved within 5–10 min.

After discharge from the hospital, all patients were advised to expose themselves to natural sunlight beginning with 15 min at least every 2 days and to increase the exposure by 5–10 min each time. All patients were able to maintain tolerance during the summer months and all were able to stay in direct noon sunlight for at least 2 h.

Pharmacological studies

The results are summarized in Table 2. Plasma histamine levels were determined at various time intervals up to 30 min after irradiation before and after induction of clinical tolerance. Prior to tolerance, the highest elevations in plasma histamine levels (7·02 and 11·8 ng/ml) occurred in patients 1 and 2 at 15 and 6 min respectively. The levels fell below 5 ng/ml by 30 min. Patient 3 showed a smaller increase in plasma histamine levels. Pre-irradiation control samples, and samples from contralateral (non-irradiated) arms in patients 2 and 3, taken between 6 and 12 min post-irradiation, contained no significant histamine (less than 5 ng/ml). In control experiments, blood taken from two normal subjects before and after similar forearm irradiation revealed no increase in plasma histamine levels following irradiation.

Following induction of clinical tolerance there was no elevation in plasma histamine in patients 1 and 3. Patient 2 developed an elevation in plasma histamine to 6·5 ng/ml at 20 min after irradiation. This increase coincided with sparse micropapular wealing over the irradiated forearm. This type of fine urticaria was frequently observed in this patient during maintenance UV-A despite failure to elicit the classic confluent solar urticarial response.

TABLE 3. Weal and flare due to histamine and codeine

	Histamine			Codeine		
Patient	Dose (μg)	Weal (area in mm²)	Flare	Dose (μg)	Weal (area in mm²)	Flare
Pre-tolerance						
1	2	110	1410	100	140	2350
				25	80	780
2	2	140	2290	100	190	2240
				25	120	950
3	2	90	1670	100	190	2330
	0·5	90	1110	25	100	1600
Post-tolerance						
1	2	110	1570	100	130	2200
				25	100	750
2	2	100	1640	100	160	2370
				25	90	1080
3	2	110	2500	100	160	3210
	0·5	80	1520	25	140	2030

Values represent area (mm²) 15 minutes after intradermal injection.

Activation of complement

In vitro irradiation of serum from solar urticaria patients did not result in a greater diminution of complement haemolytic activity than that observed in serum from normal controls. Incubation of irradiated sera at 37°C for 15 min prior to complement assay also failed to show greater consumption of complement haemolytic activity in sera of patients compared to normal sera. Immunoelectrophoretic analysis of *in vitro* irradiated serum samples from patients and normal controls showed no difference in C3 or factor B cleavage. Similar analysis of serum recovered from antecubital venous blood draining the irradiated forearm of patient 1 also did not demonstrate cleavage of C3 or factor B.

Intradermal injection of histamine and codeine

The areas encompassing the cutaneous inflammatory response following the intradermal injection of histamine and codeine into the upper back are given in Table 3. The responses before and after the induction of tolerance to UVR did not differ consistently.

Morphology

Light microscopic examination of haematoxylin and eosin stained sections revealed no detectable differences between normal and tolerant skin. Specifically, there was no hyperkeratosis, epidermal hyperplasia or increased melanin pigment. The dermis was also normal.

The patterns and degrees of mast cell changes seen by electronmicroscopy are given in Table 4. Control unirradiated specimens revealed normal intact mast cells (Fig. 1). Ultrastructural

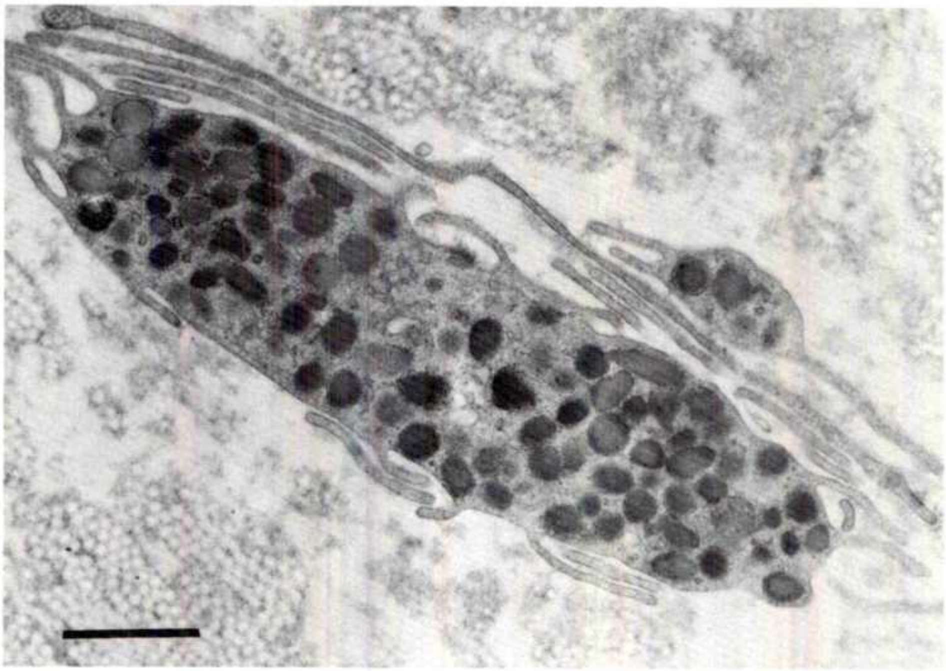

FIGURE 1. Electron micrograph of a typical resting mast cell displaying oblong shape with prominent microvillous extensions. Cytoplasm is filled with single homogeneous electron-dense granules. No evidence of granule fusion or exocytosis is seen (bar = 1 μm).

TABLE 4. Patterns of mast cell ultrastructure in skin biopsies*

Patient	Unirradiated control	Irradiated non-tolerant	Irradiated tolerant
1	0	3+	0
2	0	3+	1+†
3	0	1+	0

*See Methods section for key.
†Associated with sparse micropapular wealing.

changes during degranulation were noted in all specimens from weals provoked by UV-A prior to induction of tolerance (Fig. 2). No wealing occurred following UV-A exposure of tolerant skin in patients 1 and 3 and the mast cells appeared intact although the granules were more heterogeneous than those in control specimens (Fig. 3). Some granules possessed dark crystalline centres surrounded by a lucid amorphous material. Such 'target-like' granules were not seen in controls. Patient 2 developed sparse micropapular wealing following UV-A exposure of tolerant skin. Several mast cells were noted to be ultrastructurally altered in the papillary dermis although the preponderance of mast cells were intact with prominent granule reserves as

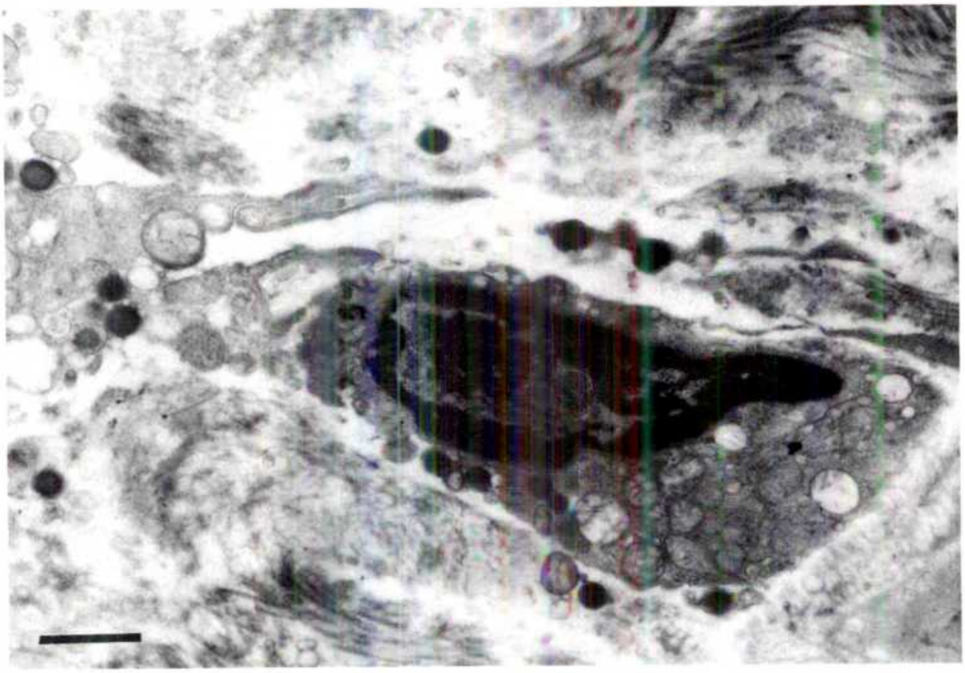

FIGURE 2. Electron micrograph of a mast cell during solar urticaria. Cytoplasm is almost devoid of granules and the mast cell lacks the characteristic oblong shape. Many granules are seen free in the dermis surrounding the mast cell (bar = 1 μm).

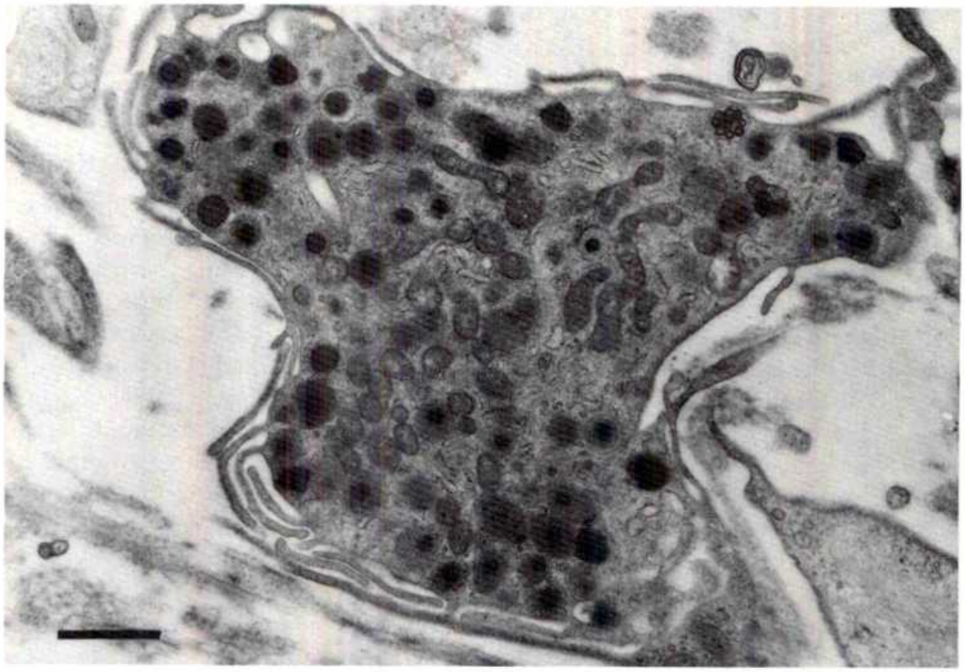

FIGURE 3. Electron micrograph of a mast cell after induction of tolerance. Cytoplasm contains single granules, some displaying a 'whorls and scroll' configuration, others with a dense inner core surrounded by a less dense material resulting in a 'target' appearance. No evidence of granule fusion or exocytosis is seen (bar = 1 μm).

noted in patients 1 and 3. There was no apparent difference in the density of mast cells between control and tolerant skin in any patients studied.

Immunofluorescence studies revealed no deposition of immunoglobulins, C3 or fibrinogen in any of the specimens from patient 1.

DISCUSSION

The induction of clinical tolerance to physical stimuli has been demonstrated in several types of urticaria including solar (Ramsay, 1977), cold (Bentley-Phillips, Kobza-Black & Greaves, 1976), cholinergic (Keahey & Greaves, unpublished observations), and localized heat urticaria (Leigh & Ramsay, 1975). Although the mechanism by which tolerance develops is unclear, one commonly held view is that repeated exposure to the offending stimulus produces degranulation of mast cells resulting in the exhaustion of mediator reserves (Bentley-Phillips *et al.*, 1976). The aim of this investigation was to test this hypothesis and evaluate other possible mechanisms by which tolerance to artificial radiation develops in patients with solar urticaria.

Although our patients differed from one another with regard to the limit of spectral sensitivity, they were all responsive to UV-A which was the waveband selected for the induction of tolerance. Complete symptomatic relief was obtained in all instances after tolerance to artificial radiation was induced. Unfortunately, the refractory state was also short-lived, as the sensitivity began to reappear within 24 to 48 h after discontinuing treatment. In order to maintain the refractory state, therefore, exposures were given every 24 h.

Once tolerance to artificial UV-A was achieved, the patients also became unresponsive to natural sunlight, which contains other shorter wavelengths (UV-B) to which two of the patients were also sensitive. Thus the induction of tolerance with one waveband may confer protection to other portions of the spectrum. This suggests that different wavelengths may act through a common mechanism to provoke urticaria in these patients. Depending on a patient's range of spectral sensitivity, UV-A or visible light would appear to be the wavebands of choice for inducing tolerance, since side-effects such as sunburn and chronic damage can be minimized.

Previous reports have established an association between histamine release and wealing in solar urticaria (Hawk *et al.*, 1980). Our results support this observation as elevated histamine levels were demonstrated in venous blood plasma draining wealed skin in three patients. Electron microscopy of irradiated skin revealed evidence of prominent mast cell degranulation. These findings are consistent with the view that the observed release of histamine derives from degranulation of cutaneous mast cells but do not necessarily implicate histamine as the primary or sole mediator. On the other hand, no elevated histamine levels were detected following similar UV-A exposures to tolerant skin in two patients and electron microscopy of irradiated-tolerant skin revealed intact resting mast cells. There was no appreciable decrease in the density of mast cells in irradiated tolerant sites, although the granules of some mast cells displayed a target-like appearance not seen in controls. Delayed histamine release and mast cell alterations were observed in one patient following irradiation of tolerant skin coincident with sparse micropapular wealing. This response was distinct from the confluent solar urticarial response observed prior to tolerance and was felt to represent partial degranulation of small numbers of mast cells beginning to lose tolerance to UVR after a 24-h interval. The inflammatory responses evoked by the intradermal injections of codeine (a mast cell degranulator) before and after induction of tolerance were similar, which further supports the microscopic findings that mast cell mediator depletion did not occur as a consequence of treatment. Intradermally injected histamine also provoked similar responses before and after tolerance, arguing against the development of cutaneous tachyphylaxis in the vascular response to histamine.

Another possible explanation for the lack of response of tolerant skin to irradiation is that epidermal changes produced by repeated exposures to UV-A will attenuate the flux of radiation reaching the dermis. Epidermal hyperplasia, hyperkeratosis, increased melanin synthesis and other changes that arise from acute exposure to UVR could alter the optical properties of the epidermis and reduce UVR transmission. Tolerance, however, can be induced within a few hours while epidermal alterations capable of affecting light transmission require 2 to 4 days to become established. Furthermore, UV-A is not noted to produce significant epidermal changes, at least at the doses employed and none were seen in the histological specimens from tolerant skin. Moreover, the MEDs were unchanged by the induction of tolerance suggesting that light transmission was not affected. Accommodation to UV-A cannot therefore be invoked as an explanation for the development of tolerance in solar urticaria.

Immunological mechanisms have been invoked for some patients with solar urticaria as illustrated in the classification proposed by Harber *et al.* (1963). These authors attempted to divide solar urticaria into six types on the basis of action spectra and the results of passive and reverse passive serum transfer experiments in human volunteers. Serum transfer studies were not performed on human volunteers before or after the induction of tolerance for ethical reasons. Nevertheless, the likelihood that UVR-induced depletion of a circulating serum factor could account for tolerance on a systemic basis seems highly unlikely in view of the observation that UV-protected sites remained very sensitive to UVR after tolerance was established.

The inability of mast cells to degranulate following induction of tolerance, despite relatively dramatic increments in UVR dose, may well relate to a direct effect on the discharge mechanism. It is conceivable that repetitive exposure to UVR renders the mast cells specifically unresponsive to stimulation by deactivating the discharge mechanism. Furthermore, this effect appears to be highly specific since tolerant skin is quite responsive to degranulating agents such as codeine. The positive response to codeine is not incompatible with this theory since there are a number of different mechanisms by which mast cell degranulation occurs *in vivo* in response to different agents (Metcalfe, Kaliner & Donlon, 1981).

Recent reports indicate that the excitation of porphyrin molecules with 405 nm radiation can cause consumption of total haemolytic complement in serum with the generation of neutrophil chemotactic products (Gigli *et al.*, 1980). These could theoretically play a role in the development of cutaneous inflammation in photosensitive patients with porphyria. A similar sequence of events could conceivably arise from the presence of another photoexcitable substance in the serum following exposure to UVR. There was no evidence, however, of complement activation in two patients whose sera were examined for this effect, and no deposition of immunoglobulins or C3 was detected by immunofluoresence in another patient.

In summary, the present findings suggest that the induction of clinical tolerance by artifical radiation in patients with idiopathic solar urticaria cannot be entirely explained by mast cell mediator depletion, histamine tachyphylaxis, an alteration in the transmission characteristic of the epidermis or by a systemic effect. It is likely that tolerance may be due to a transient and specific increase in the threshold for mast cell degranulation by UVR.

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Human skin aryl hydrocarbon hydroxylase

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SUMMARY

Aryl hydrocarbon hydroxylase (AHH) activity has been measured in full-thickness biopsies of human skin from patients with and without psoriasis. Basal levels of enzyme activity varied over a fivefold range and were not related to age, sex or clinical condition. Induction of AHH activity by the application of coal tar resulted in a two- to fivefold increase in activity above basal levels, which was not related to the presence or absence of psoriasis. Separation of human skin into dermis and epidermis showed that human skin AHH activity is predominantly dermal.

Aryl hydrocarbon hydroxylase (AHH) is a microsomal cytochrome P-450 dependent mono-oxygenase responsible for the metabolism of polycyclic hydrocarbons to more hydrophilic products (Pelkonen & Nebert, 1982). Although the liver is the primary organ of mono-oxygenase activity AHH is present and inducible in other organs. The potential importance of cutaneous AHH activity in the metabolism of drugs and carcinogens (Pelkonen & Nebert, 1982), and our inability to detect it in human epidermal suction blisters (Finnen *et al.*, 1983), have led to a reinvestigation of AHH activity in human skin using full-thickness biopsies.

METHODS

Twelve patients with psoriasis (seven male, five female, aged 16–62 years) and ten without psoriasis (five male, five female, aged 24–65 years) were studied. Coal tar solution (BPC) was applied to clinically unaffected skin on the medial aspect of the upper arm or on the abdomen. Eighteen to twenty-four hours later an elliptical biopsy approximately 1 × 3 cm was taken from the treated area and from a corresponding untreated site. AHH activity was measured in whole skin homogenates as previously described (Bickers & Kappas, 1978).

In four experiments skin samples were obtained following mastectomy and amputation (one breast, three legs). The samples were split into dermis and epidermis plus as thin a layer of dermis as possible using a Humby grafting knife. Histological examination of the separated skin showed that the epidermis was attached to a thin layer of dermis. AHH activity was determined in whole homogenates of dermis and epidermis.

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RESULTS

Basal and induced levels of AHH activity in whole homogenates of clinically unaffected skin biopsies are shown in Table 1. Basal levels of enzyme activity varied over a fivefold range in both groups of patients and the variation was not related to age, sex or nature of skin disease. Induction of AHH by coal tar application resulted in a two- to fivefold increase in activity above basal levels. The relative increase in activity caused by treatment with coal tar was not related to basal level of activity, age, sex or the presence or absence of psoriasis.

Values for AHH activity in human dermis and epidermis are shown in Table 2. Although the separation technique used results in some contamination of the epidermis with dermis the concentration of AHH activity on a wet weight basis was comparable in both dermis, epidermis and whole skin. However, total AHH activity in the dermis was five times greater than in the epidermis.

TABLE 1. Basal and induced levels of AHH activity in biopsy samples from psoriatic and normal individuals

	AHH activity			
	Normal (n = 10)		psoriasis (n = 12)	
	pmol/min/g tissue	pmol/min/cm^2	pmol/min/g tissue	pmol/min/cm^2
Basal	0·43 ± 0·09	0·018 ± 0·004	0·38 ± 0·09	0·016 ± 0·004
Induced	0·86 ± 0·10	0·036 ± 0·004	1·00 ± 0·10	0·042 ± 0·003

Results are shown as the mean ± s.d.

TABLE 2. AHH activity in human dermis and epidermis

		AHH activity	
		pmol/min/cm^2 whole skin	pmol/min/g tissue
Dermis	(4)	0·016 ± 0·004	0·37 ± 0·06
Epidermis	(4)	0·002 ± 0·001	0·41 ± 0·05

DISCUSSION

The present findings show that AHH activity is measurable in full-thickness biopsies of human skin. However, because of the relative insensitivity of existing methods, the measurement requires biopsies of the order of 50–100 mg wet weight, equivalent to 1 cm^2 whole skin or 8 cm^2 of pure epidermis. The low level of total activity together with our finding that human skin AHH is predominantly dermal explains why AHH activity cannot be measured in the relatively small amounts of epidermal tissue obtained from suction blisters (Finnen *et al.*, 1983), despite previous claims to the contrary (Chapman *et al.*, 1980; Chapman, Rawlins & Shuster, 1979a, b). The explanation for this discrepancy has been given elsewhere (Finnen *et al.*, 1983). Our present finding that AHH activity is located principally in the dermis is in agreement with our findings for mouse skin (Finnen & Shuster, in preparation) and those of Wiebel, Leutz & Gelboin (1975). In the present study we found that AHH activity was induced two- to fivefold by a single application of coal tar preparation used therapeutically. Whether this induction is in any way related to the therapeutic action of coal tar is unclear, but it does reopen the question of why skin cancer has not been noted after such therapy (Shuster, Chapman & Rawlins, 1979). The wide range of basal AHH activity in human skin (Table 1) may in part be due to previous exposure of the skin to inducing agents. There were no significant differences in either basal or induced levels of AHH activity in skin biopsies from patients with and without psoriasis, thus confirming our refutation (Finnen *et al.*, 1983) of previous suggestions of an abnormality of AHH in psoriasis (Chapmen *et al.*, 1980; Chapman *et al.*, 1979a, b; Shuster *et al.*, 1979; Shuster *et al.*, 1980, 1981; Rawlins *et al.*, 1980; Kersey *et al.*, 1981).

Given the values for human skin AHH obtained in the present study, and taking a total skin weight of approximately 4 kg corresponding to an area of 1·7 m^2, the total capacity of human skin AHH is in the order of 2 nmol/min. In comparison the total human liver activity based on a specific activity of 400 pmol/min/g (Finnen *et al.*, 1983), and an average liver weight of 1·5 kg, is 600 nmol/min. It seems unlikely therefore that human skin plays a role in the systemic metabolism of drugs, although human skin AHH could nevertheless play a part in the therapeutic efficacy and toxicity of topical drugs. In addition to drugs, it is becoming increasingly evident that endogenous lipids, steroids and prostaglandins also serve as substrates for the mono-oxygenase system (Capdevilla *et al.*, 1981). The finding of AHH activity in human skin may be indicative of a role for mono-oxygenase activity in the metabolism of endogenous lipophilic substrates both in the dermis and the epidermis.

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Pharmacology and Treatment

Inflammatory responses in acne patients treated with 13-*cis*-retinoic acid (isotretinoin)

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SUMMARY

Twelve patients with acne vulgaris treated with isotretinoin for 4 months showed increased levels of immunoglobulins and helper T cells at 8 weeks and increased levels of B cells at 16 weeks.

13-*cis*-retinoic acid (13-cRA) is an effective treatment for severe acne with the benefit of long remission periods (Peck *et al.*, 1979; Jones *et al.*, 1983). Despite the fact that this drug has recently been released for prescription by dermatologists, little is known of its mode of action.

Recently we had the opportunity to investigate eight males and five females receiving 0·5 mg/kg per day 13-cRA (isotretinoin) for 4 months. We measured the lymphocyte subpopulations, using the OKT series of monoclonal antibodies, monocyte and neutrophil numbers and C3, IgG, IgM, IgA, α_2 macroglobulin and CRP levels (radial immunodiffusion assays). Measurements were made prior to treatment and at 4-weekly intervals (Tables 1 and 2).

A continual decrease in acne severity was recorded, which agrees with previous studies. The other results show that the immune system of these patients has been stimulated at 8 weeks of therapy, as evidenced by increased levels of immunoglobulins and helper T cells ($OKT4^+$). This stimulation of T cells was followed by significantly increased numbers of B cells with surface immunoglobulin markers at 16 weeks.

Vitamin A, from which 13-cRA is derived, has been reported as an immunopotentiator (Jurin & Tannock, 1972) and other synthetic retinoids are known to possess adjuvant properties (Cohen & Cohen, 1973). Our results could be due to a direct effect of 13-cRA or an indirect effect of the antigens involved in acne. The marked increase in IgG levels at 8 weeks would suggest that these patients have been re-exposed to greater levels of antigens than those to which they have responded previously. Micro-organisms and/or their exocellular products could fit such a role. Increased exposure to these antigens may have occurred as a consequence of greater permeability of the follicular wall due to loss of epidermal cohesion (Elias *et al.*, 1981; Jones *et al.*, 1982).

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TABLE 1. Changes in inflammatory responses and acne grade during therapy

	Week				
	0	4	8	12	16
Total lymphocytes × 10^9/l	1·87	2·03	2·09	2·11	1·99
Total T cells (OKT3$^+$) × 10^9/l	1·44	1·51	1·62	1·53	1·36
Helper T cells (OKT4$^+$) × 10^9/l	0·93	1·05	1·17	1·06	0·91
Suppressor T cells (OKT8$^+$) × 10^9/l	0·51	0·51	0·43	0·47	0·43
B cells surface Ig × 10^9/l	0·35	0·41	0·37	0·36	0·54
B cells surface IgM × 10^9/l	0·15	0·21	0·21	0·20	0·27
Neutrophils × 10^9/l	4·16	3·58	3·76	3·71	3·84
Monocytes × 10^9/l	0·30	0·45	0·31	0·49	0·39
IgG (i.u./ml)	138	144	174	134	139
IgA (i.u./ml)	138	152	177	145	149
IgM (i.u./ml)	183	188	225	197	200
CRP (mg/l)	3·28	2·50	1·88	5·23	1·26
α_2 macroglobulin (mg%)	291	280	355	325	324
C3 (mg%)	173	158	170	156	154
OKT4$^+$/OKT8$^+$ ratio	2·04	2·30	2·94	2·41	2·15
Acne grade	6·0	3·3	2·9	1·9	1·0

TABLE 2. Significant changes in acne grade* and inflammatory responses during therapy

	Week 8	Week 12	Week 16
OKT4$^+$ × 10^9/l	+ ($P < 0·02$)		
OKT4$^+$/OKT8$^+$	+ ($P < 0·01$)		
B cells Sm Ig × 10^9/l			+ ($P < 0·005$
B cells Sm IgM × 10^9/l			+ ($P < 0·02$
IgG (i.u./ml)	+ ($P < 0·005$)	− ($P < 0·05$)	
IgA (i.u./ml)	+ ($P < 0·05$)	+ ($P < 0·02$)	
C3 (mg%)		− ($P < 0·05$)	− ($P < 0·02$)
α_2 macroglobulin (mg%)	+ ($P < 0·005$)		
Acne grade	− ($P < 0·01$)	− ($P < 0·01$)	− ($P < 0·01$)

+ = significantly increased.
− = significantly decreased.
* Wilcoxon's test for pair differences.
At 4 weeks only acne grade significantly altered (see Table 1).

A reduction in inflammatory mediators was evident at 12 weeks of therapy, with a small drop in neutrophil numbers, a significant decrease in IgG and a significant reduction in C3 to a level similar to that found in control subjects without acne. According to King *et al.* (1982) the numbers of anaerobic bacteria and *Pityrosporum* spp. had decreased significantly by 12 weeks of therapy. This decrease in exposure to microbial antigens would reduce some of the factors involved in inflammation and fit well with the decreased levels of the parameters measured.

This investigation suggests that there is some initial stimulation of the immune system in acne patients treated with 13-cRA which may be a contributory factor to their marked clinical improvement. However the results would not implicate 13-cRA as a potent immuno-stimulator.

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PUVA treatment of scleromyxoedema

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SUMMARY

Treatment of a 60-year-old woman with scleromyxoedema with 8-methoxypsoralen and UV-A radiation (PUVA) resulted in significant clinical and histological improvement but no change in circulating paraprotein.

Scleromyxoedema (lichen myxoedematosus, papular mucinosis) is a rare disorder, associated with paraproteinaemia, in which proliferation of fibroblasts and dermal mucinous infiltration cause characteristic cutaneous changes of diffuse skin thickening in association with confluent groups of lichenoid papules.

Various methods of treatment have been tried and have resulted in variable clinical responses. We here report a case which responded to PUVA therapy.

CASE REPORT

In 1982 a 60-year-old woman presented with an 18-month history of a papular eruption, initially confined to the knees and forearms, that later spread proximally to involve the trunk. Since the age of 30 years she had suffered with asthma requiring treatment with infrequent courses of oral steroids. Since 1971, when a diagnosis of hypothyroidism was made, she had taken thyroxine sodium 100 μg/day. On examination, the only abnormal findings were confined to the skin. An eruption composed of small, densely grouped papules on diffusely thickened, erythematous skin was present over the arms, upper legs, back and, to a lesser extent, the cheeks.

The following investigations were normal: haemoglobin, white cell count, erythrocyte sedimentation rate, blood urea and electrolytes, tests of liver and thyroid function. Serum albumin was 46 g/l and serum globulin 34 g/l.

Protein immunoelectrophoresis demonstrated an IgG lamda monoclone that was quantified at 7 g/l. No Bence–Jones proteinuria was detected, a radiographic skeletal survey was normal, and a bone marrow aspirate and trephine biopsy demonstrated no increase in plasma cells. The diagnosis of scleromyxoedema was confirmed by skin biopsy which showed an extensive fibroblast proliferation in the upper dermis with strongly positive Alcian blue staining in this area (Fig. 1). A focal lymphocytic infiltrate surrounding blood vessels was present and

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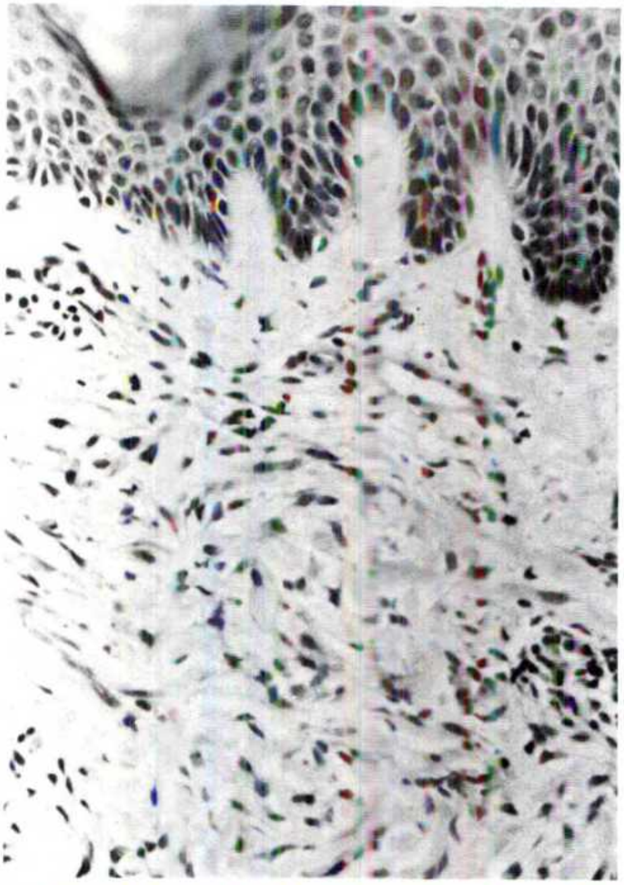

FIGURE 1. Scleromyxoedema. Biopsy of involved skin prior to treatment (H & E, × 640).

secondary pigmentary incontinence noted. Direct immunofluorescence showed no deposition of immunoglobulins (IgG, IgM, IgA and IgE), complement fraction C3, nor fibrinogen.

Treatment was commenced with cyclophosphamide at a daily oral dose of 150 mg that was later reduced to 50 mg. One month after diagnosis the patient developed repeated transient episodes of drowsiness associated with expressive dysphasia and preceded by frontal headaches. A computed tomogram and electroencephalogram were normal. No cause was found for these episodes which resolved spontaneously and have not recurred. After 5 months, treatment with cyclophosphamide was stopped, there having been no improvement in the extent, nor change in the nature, of the eruption.

In October 1982 treatment with PUVA was commenced. After 2 months an improvement in the degree of skin thickening was noted and by 4 months, after a total UV-A dose of 160 J/cm², the papular eruption had resolved completely, leaving minimal thickening of the previously involved skin. PUVA therapy has been continued (4·5 J/cm² weekly) and the clinical improvement has been maintained.

Repeat biopsies were taken from the skin adjacent to the previous biopsy sites. Histology (Fig. 2) demonstrated diminished density of fibroblasts and mucopolysaccharide stains were

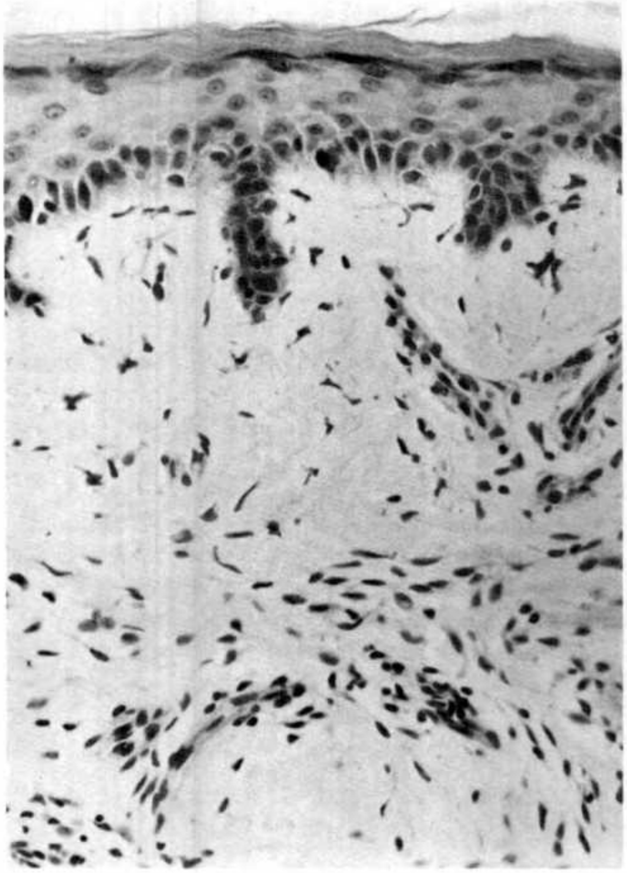

FIGURE 2. Biopsy of adjacent area after treatment with PUVA (H & E, × 640).

negative. Repeat protein immunoelectrophoresis showed the continued presence of the IgG lamda monoclone at a concentration of 6 g/l.

DISCUSSION

A variety of drugs including cyclophosphamide, melphalan and prednisolone have been suggested as forms of treatment for this disease. It has been argued (Chanda, 1979) that the use of potentially dangerous drugs such as these is not justified in a disease which, although it may be cosmetically distressing, generally has a good prognosis. Other authorities (Bleehen, 1979), however, regard the prognosis as poor and there have been several reports of death occurring at a relatively early age from myocardial infarction or cerebrovascular disease (Rudner, Mehregan & Pinkus, 1966; Lai A Fat *et al.*, 1973; Lowe *et al.*, 1982).

Superficial X-ray therapy was found, by chance, to benefit one patient (Hill, Crawford & Rogers, 1976) and a recent report (Lowe *et al.*, 1982) has demonstrated, in one case, the effectiveness of the whole-body electron beam irradiation.

We have been unable to find any previous reference to PUVA treatment of scleromyxoedema and suggest that this is a relatively safe form of therapy which may be of some use in patients with disfiguring or functionally disabling skin lesions. It is known that UV-A irradiation causes increased DNA pyrimidine dimer formation in cultured human fibroblasts (Carrier, Snyder & Regan, 1982) and that *in vivo* a single dose of UV-A causes electron microscopic nuclear and cytoplasmic changes in fibroblasts located in the subpapillary dermis (Kumakiri, Hashimoto & Willis, 1977). It seems likely that PUVA therapy inhibits either replication or function of the abnormal fibroblasts in scleromyxoedema.

The finding of an IgG monoclonal protein, usually composed of lambda light chains, is now well established and has been suggested as a necessary diagnostic criterion for scleromyxoedema (Hill *et al.*, 1976). Detection of the abnormal protein may, however, not be possible for some years after the appearance of the cutaneous lesions (Lowe *et al.*, 1982) and, conversely, the protein may persist for some years after clinical resolution of the disease (Hardie *et al.*, 1979).

The significance of the paraprotein is not yet established but it does not appear to be the only serum abnormality. *In vitro* stimulation of [^{3}H]thymidine uptake by human fibroblasts has been demonstrated using whole blood serum from patients with scleromyxoedema but no stimulation was found using the purified IgG fraction alone (Harper & Rispler, 1978).

Unexplained neurological abnormalities have been a feature of some cases (McCuistion & Schoch, 1956; Findlay & Simpson, 1960; Lai A Fat *et al.*, 1973; Lowe *et al.*, 1982). Symptoms have varied in severity from headaches with transient disturbance in speech to rapid, progressive mental deterioration with death following *grand mal* convulsions and coma. Hyperviscosity was not implicated in the most recent report (Lowe *et al.*, 1982) nor in our patient. Cerebral haemorrhage and infarction has been demonstrated at post mortem (Perry, Montgomery & Stickney, 1960; Lai A Fat *et al.*, 1973) but mucopolysaccharide infiltration of the brain has not been described.

ACKNOWLEDGMENTS

We would like to thank Dr A.M.Crone and Dr D.Birkett for their help with this case.

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Brief Communication

Dyes and preservatives in oral antihistamines

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In May 1980 we drew attention to the potential hazards associated with the inclusion of tartrazine and other additives in many commonly prescribed antihistamines (Aldridge, Main & Smith, 1980). New products have since been introduced and several drug formulations altered. To reflect these changes a fresh summary is presented.

The mechanism(s) of the adverse reaction(s) remains unexplained. The exacerbation of chronic urticaria by aspirin (James & Warin, 1970) has been confirmed and shown to extend to tartrazine, benzoates and several anti-inflammatory drugs (Doeglas, 1975). Certain characteristics of these reactions suggest a non-immune basis (Yurchak, Wicher & Arbesman, 1970; Doeglas, 1975; Warin, 1983) and prostaglandin inhibition has been proposed (Doeglas, 1975).

The modification of the arachidonic acid cascade by aspirin and certain non-steroidal anti-inflammatory drugs is known to profoundly affect certain allergic reactions; asthma can for example be exacerbated (Szczeklik *et al.*, 1976; Kay, 1982) whereas some food allergy problems may benefit (Buisseret *et al.*, 1978). Despite the known cross-sensitivity of aspirin-sensitive asthmatics to tartrazine (Settipane & Pudupakkam, 1975), attempts to identify an analogous modification of the cascade by tartrazine have been unsuccessful (Gerber *et al.*, 1979).

The possibility of some adverse affects being immunologically based has been reviewed by Miller (1982) who concluded that the immunological mechanisms involved are not those usually concerned in food and drug mechanisms. At the present time the only method of diagnosing sensitivity remains a therapeutic trial and clearly avoidance of unnecessary exposure to potential allergens remains desirable.

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TABLE 1 Dyes and preservatives in antihistamines

Group	Active ingredient	Manufacturer	Preparation	Dosage form	Dye	Preservative
Alkylamine	Brompheniramine maleate	A.H Robins	Dimotane●	Elixir	Caramel Amaranth (E123)	Sodium benzoate
				L A. tablets	Titanium dioxide Iron Oxide Yellow Iron Oxide Red Opacode Black	Nil
				Tablets	Iron Oxide Yellow Iron Oxide Red	Nil
	Chlorpheniramine maleate	Allen & Hanburys Ltd	Piriton●	Tablets	Yellow Iron Oxide	Nil
				Spandets	Yellow Iron Oxide	Nil
				Duolet	Yellow Iron Oxide Titanium dioxide	Benzyl benzoate
				Syrup	Nil	Nipasept (contains benzoate)
	Dimethindene maleate	Zyma	Fenostil retard●	Tablets	Titanium dioxide	Nil
	Pheniramine maleate	Hoechst	Daneral S.A ●	Tablets	Erythrosine Iron Oxide Yellow	Nil
Ethanolamine	Diphenylpyraline hydrochloride	Riker	Lergoban●	Tablets	Nil	Nil
	Diphenhydramine hydrochloride	Parke Davis	Benadryl●	Capsules	Erythrosine Titanium dioxide	Nil
				Cream 2% (discontinued)	Nil	Methylhydroxybenzoate
	Diphenhydramine hydrochloride	Parke Davis	Caladryl●	Cream	Nil	Propylhydroxybenzoate
	Diphenylpyraline hydrochloride	Smith Kline and French	Histryl●	Spansule	Amaranth (pellets) Erythrosine (shell)	Nil
	Diphenylpyraline hydrochloride	Smith Kline and French	Histryl paediatric●	Spansule	Amaranth (pellets) Erythrosine (shell)	Nil
Ethylenediamine	Mepyramine	May and Baker	Anthisan●	Tablets	Brilliant Blue FCF	Nil

(*cont.*)

TABLE I. (*cont.*)

Group	Active ingredient	Manufacturer	Preparation	Dosage form	Dye	Preservative
Phenothiazine	Mequitazine	Smith and Nephew	Primalan®	Tablets	Nil	Nil
	Dimethothiazine	May & Baker	Banistyl®	Tablets	Nil	Nil
	Promethazine hydrochloride	May & Baker	Phenergan®	Elixir	Amaranth Tartrazine	Nil
				Tablets	Brilliant Blue FCF	Nil
	Trimeprazine tartrate	May & Baker	Vallergan®	Syrup	Tartrazine	Methylhydroxybenzoate
				Syrup Forte	Amaranth	Methylhydroxybenzoate
				Tablets	Brilliant Blue FCF Erythrosin	Nil
Piperazine	Hydroxyzine hydrochloride	Pfizer	Atarax®	Tabs. 10 mg	Dispersed Orange (Anstead 11348)	Nil
				Tabs. 25 mg	Tartrazine Acid Brilliant Green	Nil
				Syrup	Nil	Sodium benzoate
Propylamine	Triprolidine hydrochloride	Wellcome	Actidil®	Tablet	Nil	Nil
				Elixir	Sunset Yellow FCF	Methylhydroxybenzoate plus sodium benzoate
	Triprolidine hydrochloride	Wellcome	Pro-Actidil®	Tablet	Indigo Carmine Lake Dispersed Erythrosine Lake Dispersed	Nil
Others	Astemizole	Janssen	Hismanal®	Tablet	Nil	Nil
	Azatadine maleate	Warrick	Optimine®	Tablet	Nil	Nil
				Syrup	FD & C Yellow 6	Methyl and propyl parabenzoate
	Clemastine hydrogen fumarate	Wander	Tavegil®	Tablets	Nil	Nil
				Elixir	Nil	Methylhydroxybenzoate Propylhydroxybenzoate
	Cyproheptadine hydrochloride	Merck Sharp and Dohme	Periactin®	Tablets	Nil	Nil
				Syrup	Quinoline Yellow E.104 (D and C No. 10)	Sorbic acid

TABLE I. (*cont.*)

Group	Active ingredient	Manufacturer	Preparation	Dosage form	Dye	Preservative
	Mebhydrolin napadisylate	Bayer	Fabahistin®	Tablet	Yellow Orange S (coat only)	Nil
				Suspension	Yellow Orange S	Methyl and propyl hydroxybenzoate
	Oxatomide	Janssen	Tinset®	Tablet	Nil	Nil
	Phenindamine	Sinclair Pharmaceuticals	Thephorin®	Tablets	Titanium dioxide	Nil
	Brompheniramine maleate Phenylephrine hydrochloride Phenylpropanolamine hydrochloride	A.H.Robins	Dimotapp LA®	Tablets	FD & C Red Lake (E127) FD & C Blue Lake (E132) Titanium dioxide (E171) Iron oxide (E172) Opacode White	Nil
	As above	A.H.Robins	Dimotapp®	Elixir	Caramel	Sodium benzoate
	Triprolidine hydrochloride Pseudoephedrine hydrochloride	Wellcome	Actifed®	Tablets	Nil	Nil
				Syrup	Tartrazine	Methylhydroxybenzoate plus sodium benzoate
	As above plus codeine	Wellcome	Actifed compound linctus®	Syrup	Amaranth	Methylhydroxybenzoate plus sodium benzoate
	Pseudoephedrine hydrochloride Triprolidine hydrochloride Codeine phosphate Guaiphenesin	Wellcome	Linctus adult expectorant®	Syrup	Sunset Yellow FCF	Methylhydroxybenzoate plus sodium benzoate
	As above	Wellcome	Linctifed paediatric expectorant®	Syrup	Ponceau 4R	Methylhydroxybenzoate plus sodium benzoate
	Terfenadine	Merrell	Triludan®	Tablets	Nil	Nil
			Triludan®	Suspension	Nil	Methylhydroxybenzoate sodium Propylhydroxybenzoate sodium Benzyl alcohol

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Case Reports

The association of keratosis pilaris atrophicans and woolly hair, with and without Noonan's syndrome

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SUMMARY

We describe two cases in which keratosis pilaris atrophicans faciei (ulerythema oophryogenes) occurred in association with hereditary woolly hair. This association also occurred in the father of case 1. In case 2, Noonan's syndrome was also present.

Both keratosis pilaris atrophicans faciei (ulerythema oophyrogenes) and hereditary woolly hair are well recognized though uncommon skin disorders, thought to be inherited as autosomal dominant traits. Several other conditions have been reported to be associated with each disease, but this is the first account of these two conditions occurring together.

CASE REPORTS

Case 1

This boy was noted at the age of 6 months to be developing extremely curly scalp hair. Soon afterwards, patches of red rough skin appeared in the supraorbital region obliterating his eyebrows, and the skin on his cheeks became similarly affected. The facial eruption then continued to spread until both sides of his face and his ears were extensively affected. On presentation he was a 5-year-old boy of normal stature and physical development. The supraorbital skin showed keratin plugs in the follicles and marked erythema of the surrounding skin with loss of eyebrow hair. His cheeks and ears were also strikingly involved (Fig. 1). There was mild typical keratosis pilaris of the upper arms and thighs. His scalp hair was light brown, short and tightly curled giving a 'Negroid' appearance. The rest of his body hair was normal.

Investigations. Histological examination of a punch biopsy from the left eyebrow showed a distorted hair follicle plugged with keratin and absent sebaceous glands. There was a sparse lymphocytic infiltrate in the dermis.

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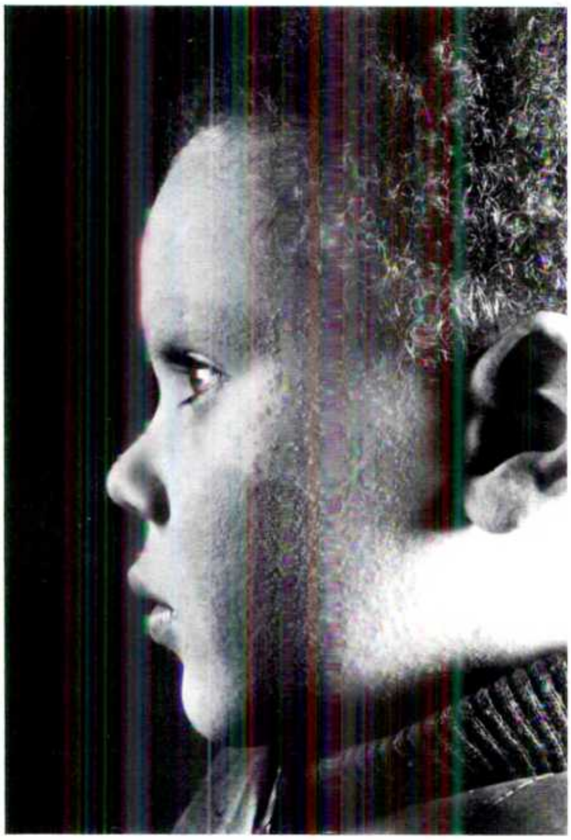

FIGURE 1. Case 1. Prominent follicular plugs on cheeks and eyebrows. Tightly curled hair also shown.

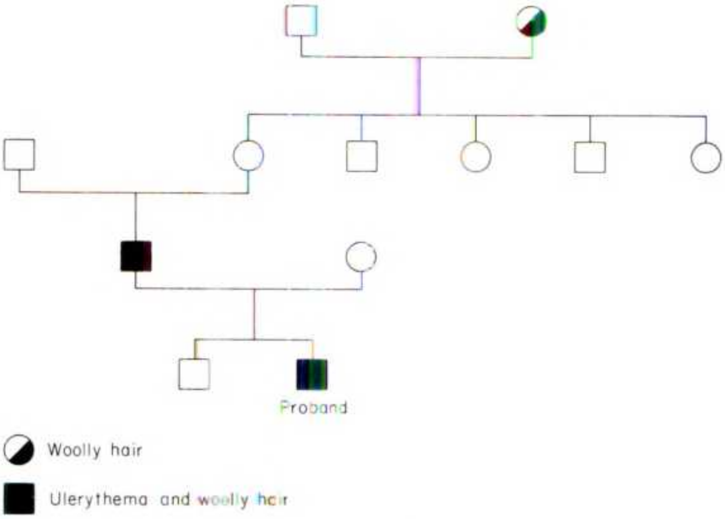

FIGURE 2. Pedigree of case 1.

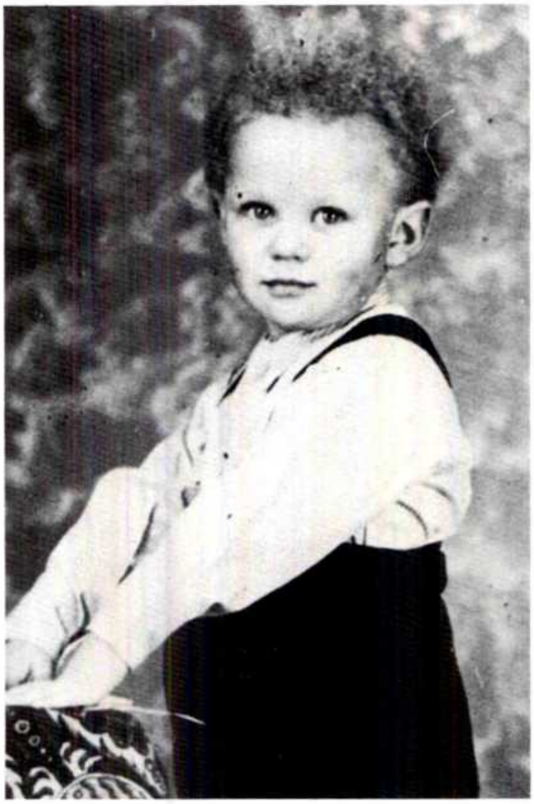

FIGURE 3. Father of case 1 as a child. Hair tightly curled, eyebrow hairs absent, erythema and keratin plugs present on cheeks and eyebrows.

Microscopic examination of scalp hairs showed 180° axial twisting of the hair shaft, but no other abnormality.

Dermatoglyphics—positive features in favour of Noonan's syndrome included t′ or t″ triradii and a high ridge count due to the presence of large ulnar loops and whorls in the fingertips. The overall pattern was in other respects unremarkable.

Chromosome count—normal male karyotype (46 XY).

Family history (Fig. 2). The proband's only brother is unaffected. His father had both conditions in childhood (Fig. 3) apparently of similar extent and severity, but with some diminution of the curliness of his hair since early adult life, and almost complete recovery of the facial skin with only minimal atrophy and no residual keratin plugs or erythema. His paternal great-grandmother also had extremely curly hair.

Case 2

This 15-year-old girl was first noted to have rough red skin on her cheeks at the age of 3 years and to have had unusually curly hair since the age of 18 months. She was a large baby (4·6 kg) at birth and was referred to Great Ormonde Street Hospital, at age 3 months, because abnormal palmar creases and dermatoglyphics had been noted as well as marked ptosis of the left eye, and a

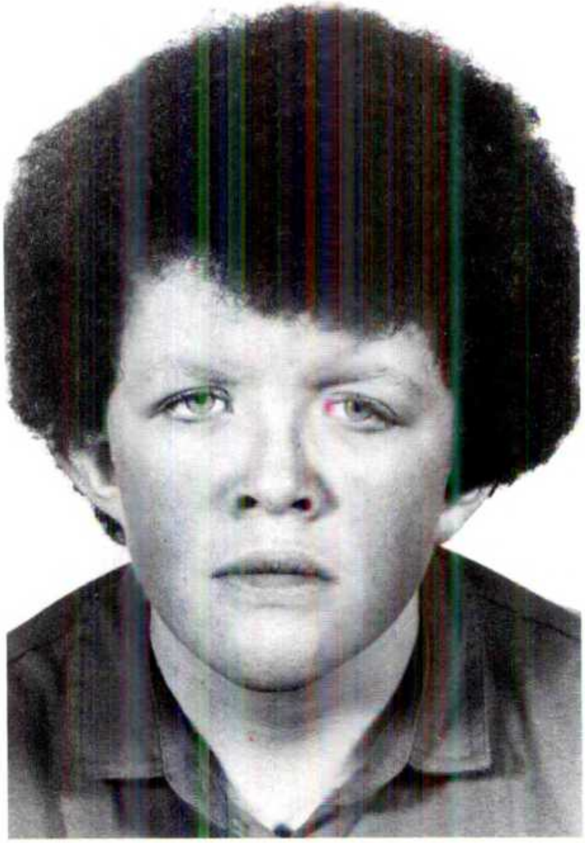

FIGURE 4. Case 2. Hypertelorism, low set ears and strikingly curly hair with low hair line. Erythema of cheeks and brow with follicular plugging.

chromosome abnormality was suspected. Chromosome studies and her subsequent development were normal, but because of a systolic murmur suggestive of mild pulmonary stenosis and her unusual facies, a diagnosis of Noonan's syndrome was made. On examination she was a healthy teenage girl with strikingly curly brown hair, ulerythema oophryogenes over the cheeks, chin and supraorbital ridges and scanty eyebrow hair (Fig. 4). Ptosis (surgically corrected), low set ears and hypertelorism were noted and she also had short broad hands with unusual skin creases and dermatoglyphics and hyperextensible joints. The skin on her body showed typical keratosis pilaris on the arms and upper thighs. There was no family history of woolly hair, keratosis pilaris or Noonan's syndrome.

DISCUSSION

Keratosis pilaris atrophicans has been recognized since 1885. It is now believed to be a congenital ectodermal defect of the pilosebaceous system and three main groups have been differentiated on the basis of associated defects. These groups may however simply represent different stages of a single process.

(i) *Keratosis pilaris atrophicans faciei* (syn. folliculitis rubra, keratosis pilaris rubra). Ulerythema oophryogenes was the name given by Taenzer to this condition in 1889 (literally—scarring erythema of the eyebrows). This term is used to describe a condition which starts at birth or in early infancy and is more or less confined to the eyebrows, although the cheeks may show similar changes. There are small horny hemispherical papules occluding the follicles surrounded by an erythematous halo. Pitted scars may result.

(ii) *Keratosis pilaris decalvans* (syn. keratosis follicularis spinulosa decalvans, follicular ichthyosis) also begins in infancy. There is more extensive keratosis pilaris, atrophy is pronounced and cicatricial alopecia develops in the scalp and eyebrows with atrophoderma of the cheeks (sex-linked recessive inheritance of this type is probable).

(iii) *Atrophoderma vermiculata* (syn. acne vermoulante, honeycomb atrophy, folliculitis ulerythematosa reticulata) begins later, between the ages of 5–12. The pre-auricular region and cheeks are affected and reticulate atrophy soon develops as the follicular plugs are shed. Milium formation is also characteristic. The inheritance of this group and of ulerythema oophryogenes is thought to be autosomal dominant.

Various associations with this group of conditions have been recorded, in particular with the sex-linked recessive type, keratosis pilaris decalvans. Dwarfism and cerebral atrophy was described in one family by Cantu *et al.* (1974), palmar plantar hyperkeratosis was recorded as an associated genodermatosis by Kuokkanen (1971), deafness was noted by Morris, Ackerman & Koblenzer (1969) and cataracts are referred to by Koolj & Venter (1959) and Franceschetti *et al.* (1956).

One recent discovery of relevance to our cases is that ulerythema oophryogenes may be a cutaneous marker for Noonan's syndrome (Pierrini & Pierrini, 1979). Noonan's syndrome is characterized by short stature, hypertelorism and occasional palpebral ptosis, moderate mental deficiency, skeletal malformations and ectopic testis, and is associated with cardiac anomalies, especially stenosis of the pulmonary artery valves. The karyotype is normal and a familial incidence is reported (Noonan, 1968). Curly hair is also said to be a feature of Noonan's syndrome (Wyre, 1978) but curly hair of the degree seen in our patients is more compatible with a diagnosis of hereditary woolly hair as classified by Hutchinson, Cairns & Wells (1974). They recognized three main types of woolly hair:

(i) Hereditary woolly hair (an autosomal dominant condition) is extremely curly hair which is normal in colour and grows at a normal rate. The hair is usually kept cut short as otherwise it is unmanageable.

(ii) Familial woolly hair is usually very pale in colour and short because it fractures easily. The inheritance is thought to be recessive.

(iii) The woolly hair naevus is a localized naevoid abnormality of a portion of the scalp, present from birth and associated with epidermal naevi on other parts of the body.

Woolly hair is said in a few cases to be associated with cataract, reduction in normal body hair and local abnormalities of the hair shaft such as trichorrhexis nodosa. No other significant associations have been described.

Our two patients showed keratosis pilaris atrophicans and hereditary woolly hair occurring together and in one case the association occurred in two generations. Either keratosis pilaris had occurred as a spontaneous mutation transmitted as an autosomal dominant trait closely linked to woolly hair, or keratosis pilaris represents a previously unrecognized association with woolly hair. The presence of Noonan's syndrome in case 2 supports the suggestion of Pierrini &

Pierrini (1979) that keratosis pilaris atrophicans faciei may be associated with Noonan's syndrome.

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Acquired cutis laxa and multiple myeloma

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SUMMARY

We report a patient with acquired cutis laxa and multiple myeloma, and the possible association between the two conditions is discussed.

Cutis laxa is a rare condition characterized by loose inelastic skin which hangs in folds. It may be congenital or acquired (Cunliffe, 1979). Apart from age of onset, the congenital form is marked by a characteristic facies with a hooked nose and long upper lip not seen in the acquired variety (Reed, Horrowitz & Beighton, 1971; Brown *et al.*, 1982).The basic pathology common to both varieties is a deficiency of elastic fibres, and organs other than the skin may be involved, resulting in emphysema, aortic aneurysm, intestinal diverticulosis and multiple hernias (Reed *et al.*, 1971; Cunliffe, 1979). The aetiology of this condition is still unclear. Our case is interesting in that the patient had multiple myeloma in addition to acquired cutis laxa.

CASE REPORT

A 45-year-old Malay female was admitted to the University Hospital in April 1982. Five years before admission she noticed intermittent puffiness of the eyelids. She consulted a doctor and was suspected to have renal oedema. Investigations at that time yielded the following results: haemoglobin 8·8 g/dl, ESR 80 mm/h, serum albumin 2·2 g/dl, globulin 7 g/dl, creatinine 1 mg/dl, urine protein 300 mg/24 h, urine red blood cells 1/mm^3 and urine white blood cells 5/mm^3. Three years later she developed progressive laxity of the skin starting from the eyelids and spreading gradually to the face, neck, trunk and perineum. Associated with this the patient noticed malaise and progressive exertional dyspnoea.

The parents were not consanguinous and there was no family history of skin disease.

On examination the patient appeared much older than her age. The skin showed marked laxity and diminished elasticity over the entire body, with loose folds and sagging, most striking on the face, neck, axillae and perineum (Fig. 1). Mild pallor was present and the chest was hyper-resonant with diminished cardiac and liver dullness. The heart was normal. A large rectocoele was present.

Investigations revealed a haemoglobin level of 7·5 g/dl, total white cell count of 3800/mm^3 and platelet count of 151,000/mm^3. The ESR was 88 mm/h. The serum albumin was 2·2 g/dl

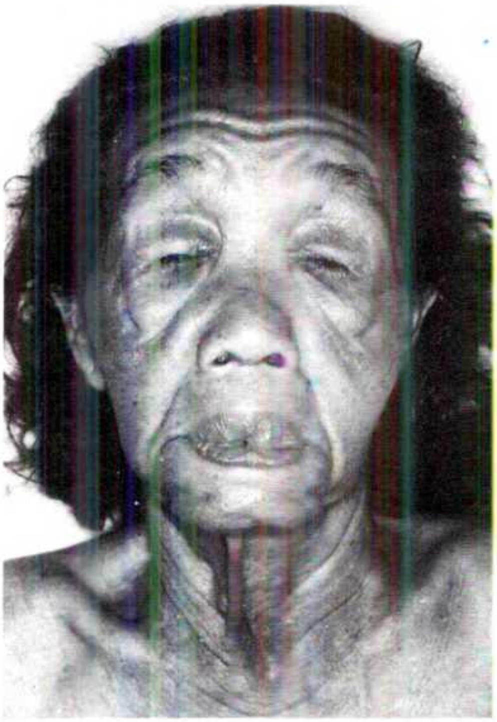

FIGURE 1. Lax inelastic skin with loose folds and sagging.

and globulin 7·5 g/dl. Bence-Jones protein was detected in the urine and serum protein electrophoresis showed a monoclonal band in the slow γ region that on immunoelectrophoresis turned out to be an IgG lambda paraprotein. The urine protein was 4·2 g/24 h and a lambda paraprotein was detected on immunoelectrophoresis as well. The serum IgG was markedly elevated (6360 mg/dl) while IgA and IgM were decreased (67 mg/dl and 68 mg/dl respectively). The bone marrow contained 57% plasma cells with immature forms showing prominent nucleoli.

The serum copper was 104 μg/dl (normal 80–150 μg/dl) and serum ceruloplasmin 140 mg/dl (normal 30–60 mg/dl).

The following tests gave normal or negative results: serum calcium, inorganic phosphate, creatinine, bilirubin, transaminases, alkaline phosphatase, anti-nuclear antibody, rheumatoid factor and complement (C3 and C4). The chest X-ray, electrocardiogram and barium meal with follow-through examination were all normal. Intravenous urogram showed a small bladder diverticulum. A bone survey showed osteolytic lesions in the skull and left humerus. Respiratory function tests showed a mild restrictive defect.

A biopsy specimen of the skin (Fig. 2a) showed absence of elastic fibre in the upper dermis. In the lower dermis the elastic fibres were markedly diminished. On electron microscopy the elastic fibres were abnormal in that elastin was diminished and loosely aggregated microfilaments were seen throughout the fibres (Fig. 2b). The collagen fibres were normal. Direct electron microscopic immunofluorescence study (using the horseradish peroxidase technique) did not show any antibodies bound to elastic fibres.

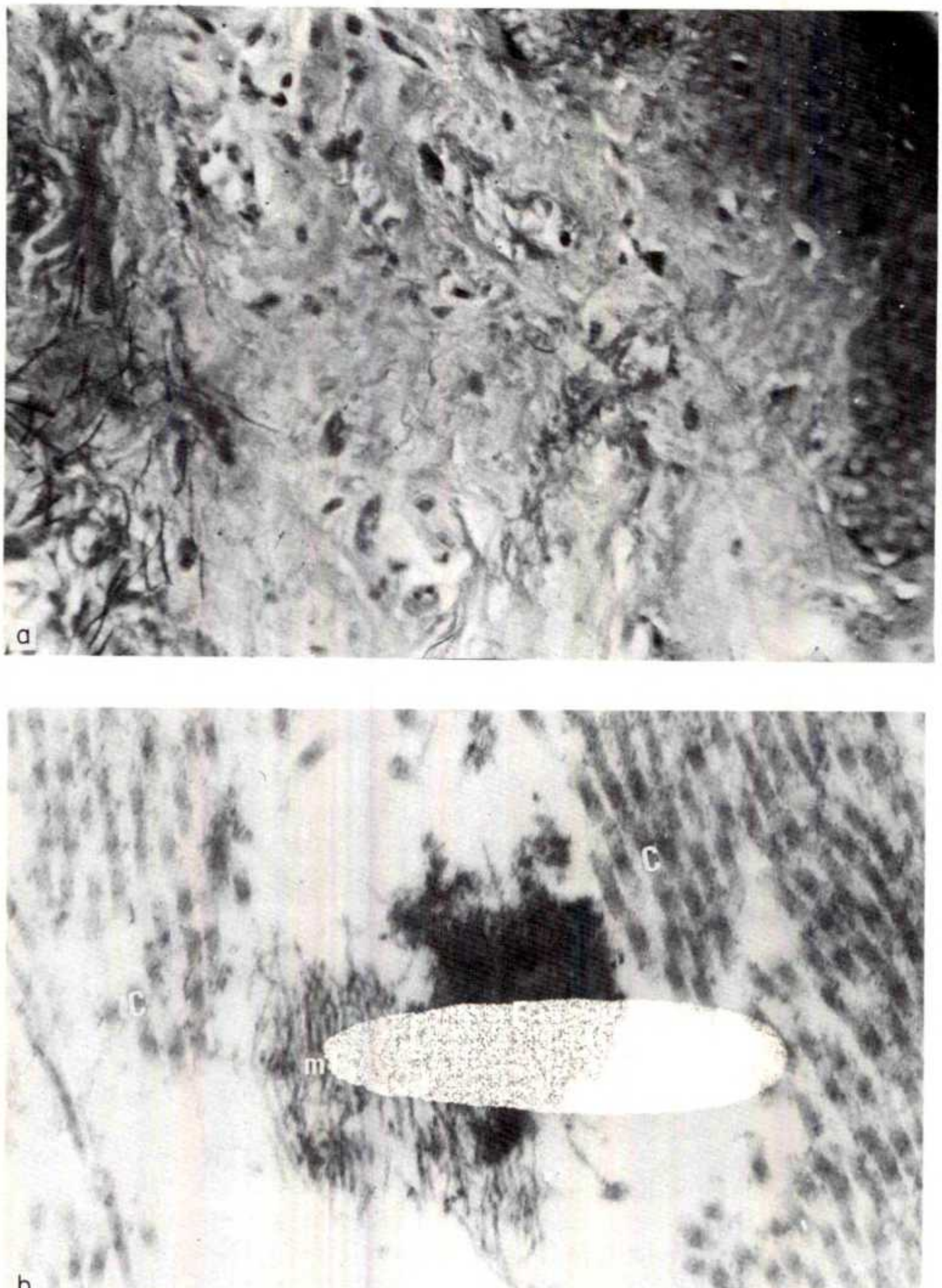

FIGURE 2. (a) Elastic fibres absent in upper dermis and markedly diminished in lower dermis (Verhoeff stain, × 250). (b) Electronmicrograph showing elastic fibres with loosely aggregated microfibrils (mf). Elastin (E), visible as amorphous material, is present only in part of the fibre. (C) Collagen. (Osmium fixation, lead citrate and uranyl acetate staining. Original magnification × 33,000, enlarged 2·1 times).

DISCUSSION

Our patient has acquired cutis laxa with systemic involvement of lungs, rectum and bladder. She also has multiple myeloma with an IgG lambda paraprotein. The multiple myeloma was diagnosed only at admission in 1982 but in retrospect the existence of anaemia, high ESR and marked hyperglobulinaemia 5 years earlier suggests that the disease was probably already present at the time when the skin changes of acquired cutis laxa first appeared.

Acquired cutis laxa is distinctly rare and there are only twenty-one well-documented cases in the literature (Reed *et al.*, 1971; Bernstein *et al.*, 1971; Thanning & Beighton, 1972; Harris, Heaphy & Perry, 1978; Nanko *et al.*, 1979; Samantry, 1981). Multiple myeloma is also not common, the incidence being 3 per 100,000 population (Kyle & Bayrd, 1976). The concurrence of two uncommon disorders such as these is probably more than coincidental. There are at least two other cases with a similar combination in the literature (Hashimoto & Kanzaki, 1975; Scott, Kauh & Luscombe, 1976) and this increases the likelihood that there is a significant association between the two conditions.

TABLE 1. Postulated causes of cutis laxa

Postulated cause	Objection
Reduced serum elastase inhibitor (Goltz *et al.*, 1965)	Not confirmed by other workers (Scott *et al.*, 1976; Harris *et al.*, 1978)
Copper deficiency (Reed *et al.*, 1971)	Normal and elevated copper levels reported (Harris *et al.*, 1978)
Decreased lysyl oxidase activity (Byers *et al.*, 1980)	(a) Patients described were atypical and may have had Ehlers–Danlos disease (Brown *et al.*, 1982) (b) Normal lysyl oxidase level demonstrated in a case (Brown *et al.*, 1982)

The pathogenesis of cutis laxa is still unclear. Table I summarizes the various suggestions together with the main objections.

Recently Nanko *et al.* (1979) have suggested that acquired cutis laxa could result from an acquired autoimmune mechanism involving the elastic fibres.

The coexistence of multiple myeloma and acquired cutis laxa attracts speculation on the possible role of humoral immunity in the pathogenesis of this disorder. It is known that human myeloma immunoglobulins may at times possess antibody activity of the autoimmune type, e.g. anti-erythrocyte, anti-IgG, anti-lipoprotein, anti-transferrin and anti-serum albumin (Vincendean *et al.*, 1980). A human immunoglobulin has recently been reported to have probable antibody activity against the basal cells of the skin as well (Vincendean *et al.*, 1980). Could the link between some cases of multiple myeloma and acquired cutis laxa lie in an immunoglobulin with anti-elastin activity?

Cho & Maguire (1980) have reported an antibody (IgG) bound to dermal elastic fibres on direct immunofluorescence in a patient with multiple myeloma and acquired cutis laxa. We were not able to confirm this finding—the electron microscopic direct immunofluoresence studies on our patient's skin did not reveal any antibody bound to elastic fibres. Scott *et al.* (1976) was also not able to demonstrate any circulating antibody to elastic fibres in a similar patient. The presence of an anti-elastin antibody in such patients thus has yet to be confirmed and further studies are necessary to determine whether the association between acquired cutis laxa and multiple myeloma does in fact have an immunological basis.

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Comment

REFLECTIONS ON HEAT

It is the prevailing doctrine that the rays of the sun that are deleterious to the skin lie in the ultraviolet (UV) region (Hausser & Vahle, 1927; Cleaver, 1970). Experimental photobiologists like Blum (1959), Magnus (1976) and Forbes (1978) have established that sunburning rays, 280–315 nm (UV-B), are photocarcinogenic. UV-A (315–400 nm), although requiring vastly higher doses than UV-B, can produce similar changes. It can induce erythema (Kaidbey & Kligman, 1979) and damage blood vessels (Kumakiri, Hashimoto & Willis, 1977; Gilchrest *et al.*, 1982), and in murine skin it has induced elastosis (Berger, Tsambaos & Kaase, 1980). UV-A also produces papillomas which may progress to squamous cell cancers (Forbes, Davies & Cole, 1982). Finally, UV-A augments the sunburning (Willis, Kligman & Epstein, 1973) and carcinogenic effects of UV-B (Willis, Menter & Whyte, 1981).

However, terrestrial sunlight is polychromatic, extending beyond the UV region into the visible (400–700 nm), infra-red (700–1,000,000 nm) and ultimately radiowaves. The latter can probably be ignored with regard to skin but visible radiation (i.e. light), seemingly innocuous, cannot. Deleterious effects ranging from phototoxic reactions (Kaidbey & Kligman, 1978) to DNA crosslinks (Gantt *et al.*, 1979) and tumour enhancement (Griffin *et al.*, 1955) have been reported. Light also causes solar urticaria in susceptible persons (Harber & Bickers, 1981). The effect of light in preventing or augmenting the injurious skin effects of UV-B and UV-A has yet to be investigated.

Curiously infra-red radiation (IR), which comprises about 40% of the irradiance reaching the earth's surface (Koller, 1965), has been largely ignored by experimental photobiologists. This neglect may stem from theoretical considerations which deter experimentation. It is a basic tenet that photochemical reactions are not heat dependent. Temperature is of no importance in the production of excited states. However, this ignores the fact that biochemical reactions are strongly heat dependent. Products of photochemical reactions might readily be amplified by heat. It is in this sense that the effects of heat could produce a marked effect on ultraviolet injury.

Heat cancers: epidemiological evidence

The precancers and cancers resulting from UV irradiation are well known. These include actinic keratoses, basal cell epitheliomas and squamous cell carcinomas. Acute heat exposure severe enough to cause ulceration can also produce squamous cell cancers (Lawrence, 1952). It is insufficiently appreciated that chronic heat exposure in the physiological range can produce the same neoplasms as UV radiation. Though mainly derived from local folklore, the evidence is convincing. In northern China, heat-induced carcinoma is known as Kang cancer. For centuries, persons in these cold regions have slept on beds of hot bricks. In India, the Kangri cancers of Kashmir arise from the habit of wearing, next to the skin, pots containing hot coals. Even in modern Japan, pocket-sized metallic benzene-burning flasks are available to maintain skin warmth in freezing weather. The tumour induced by this practice is called Kairo cancer. A fourth species of heat cancer, Turf or Peat Fire Cancer, has been trenchantly described by Cross (1967). He reported over 160 cases of cancer of the lower leg in rural Irish women who had spent many hours at chores in front of an open hearth containing burning peat. Invariably, the tumours arose in skin that showed the hallmarks of erythema ab igne.

It now appears that there is yet a fifth type of heat cancer. Unusual examples of basal cell carcinomas have been brought to our attention by Kocsard of Australia (personal communication). He has encountered two patients with basal cell cancers on the identical area of the cheeks. Both wore rimless glasses which focussed IR and light rays from sunlight on to a small region which became the site of an epithelioma (Fig. 1). This phenomenon had been reported earlier by Corson *et al.* (1949) who also showed that the focus site was approximately 3°C higher than surrounding skin. Rimmed glasses do not produce this effect.

Erythema ab igne: clinical evidence

Although dermatologists have a long familiarity with erythema ab igne, few systematic clinical and histological studies have been performed. Findlayson, Sams & Smith (1966) reviewed reports from the mid 19th century and summarized the salient histological features. The condition was fairly common in Britain before the introduction of central heating. After years of exposure to intense infra-red irradiation, keratoses often sprang up on a background of reticulated, pigmented skin. These keratoses were indistinguishable, histologically, from those that are actinically induced.

The histological similarities between chronic actinic and infra-red induced damage are striking (Kligman, 1969), and in fact, many of the changes evoked by UV can be mimicked by heat. It should be emphasized that these chronic changes are brought about by temperatures which are insufficient to produce an acute burn. Proliferation of elastic fibre is an early sign of damage from both UV radiation and heat. Increased cellularity in the dermis, especially mast cells (unpublished observations) and tortuous telangiectatic vessels are common to both. In

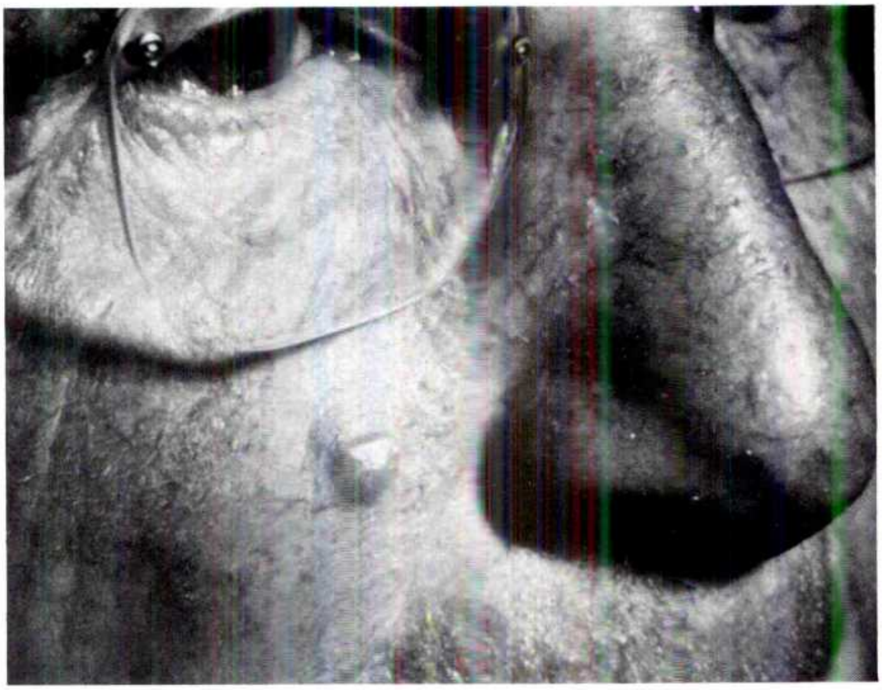

FIGURE 1. Basal cell epithelioma below rimless glasses at site of focussed solar irradiation. (Photograph through the courtesy of Emery Kocsard, M.D.)

advanced cases there is marked cellular atypia with irregularities in size, shape and staining properties of keratinocytes. Areas of dysplastic cells give rise to hyper- and parakeratotic lesions in both conditions. Melanin distribution within epidermal cells is irregular with segments of hypo- and hyperpigmented keratinocytes lying side by side (Hill & Eidenow, 1923). The presence of large amounts of melanin, as in Negro skin, raises an interesting question in connection with heat. Although Negro skin has greater absorption of IR and visible radiation (Corth, 1983) it has less elastosis than age-matched Caucasian skin (Kligman, 1969). The likely explanation for this seeming discrepancy is found in the capacity of melanin to absorb UVR, thereby preventing many photons from reaching the dermal fibroblasts. Much of the elastosis seen in Negro skin may be due to IR alone.

Although the broad pattern of epidermal and dermal changes is similar whether due to heat or UVR, there are some differences. For example, dermal deposition of haemosiderin and melanin is more prominent in erythema ab igne. Also, the hyperplasia of elastic fibre never reaches the vast, amorphous end-stage of degenerative solar elastosis. While the pathogenesis is doubtless different, the paths of chronic heat and actinic injury run parallel.

In urban America, the increased use of space-heaters, woodburning stoves and fireplaces to counter the high cost of central heating fuel has resulted in a resurgence of erythema ab igne, particularly in the elderly poor, but also in impecunious students.

Space-heating devices are not the only source of chronic IR damage. The use of a heating pad for pain relief for several hours daily and for many months can also cause erythema ab igne. Glass blowers show severe cutaneous ageing changes by early adulthood. Bakers and kitchen workers who have been exposed to high temperatures for decades have faces and arms much older looking than their chronological age (Fig. 2). They sometimes show telangiectasia and the reticulated rash suggestive of erythema ab igne. Elastosis is common. The baleful effects of chronic heat exposure have scarely been noticed as an occupational hazard.

Even the seemingly innocuous hair dryer may be harmful. We saw a striking example of ruined skin in a 30-year-old woman who after washing her hair daily, used a hood-type dryer at a high setting for 1·5 hours daily for 7 years! The dire results were twin pouches of lax, inelastic skin sagging downwards on both cheeks (Fig. 3).

A new trend in U.S.A. is to dry the hair by sitting beneath three or four naked IR bulbs for 20 to 30 minutes. These shine on the face as well as on the hair. Extrapolation from our experimental observations would predict that this will cause substantial elastosis. It will be interesting to see whether this practice will reproduce changes which support the suggestion of O'Brien (1978) that IR may predispose to temporal arteritis.

It appears that whether heat reaches the skin by conduction (i.e. hot bricks, heating pads) or by radiation (i.e. open fires, space heaters), the changes are quite similar. Cancers and erythema ab igne can be produced via either route.

Experimental studies

Although the harmful effect of heat is ancient knowledge, as noted by Schwartz (1978), experimenters seem not to have become intrigued. The few early studies combining heat with UV radiation suffer from inadequate methods and the data are conflicting with regard to amplification of acute UV injury (Clark, 1931; Everett *et al.*, 1963). Kaidbey, Witkowski & Kligman (1982) have been unable to demonstrate that intense IR exposure for 30 minutes before, during or after UV irradiation (or all three together) lowers the human minimal erythema dose (MED). Even the severe reactions evoked by six MEDs are not exacerbated by heat.

The possibility of enhancement of chronic actinic damage by IR radiation is of greater

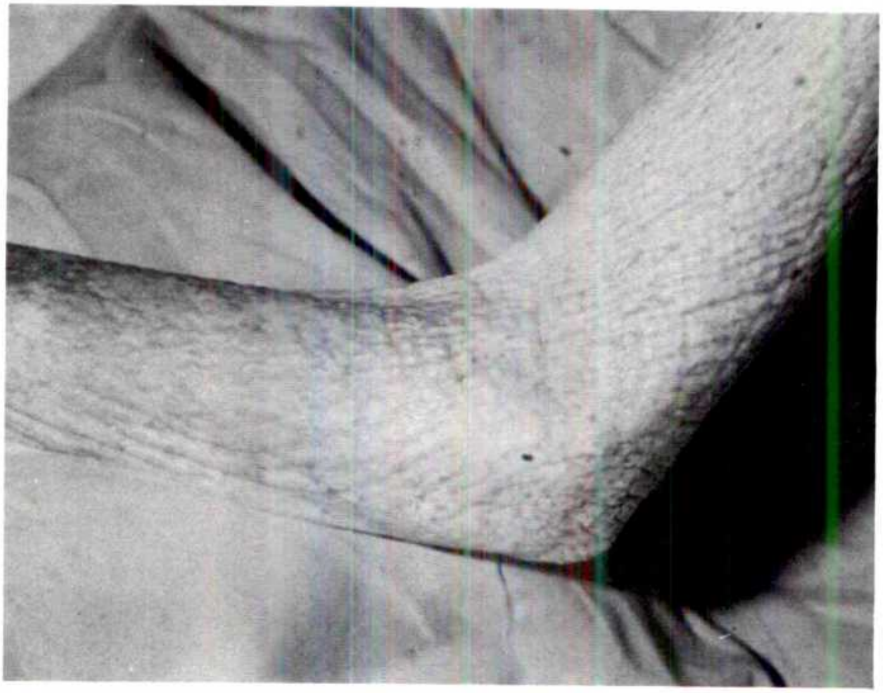

FIGURE 2. Thickened, leathery elastotic skin on arm of kitchen worker.

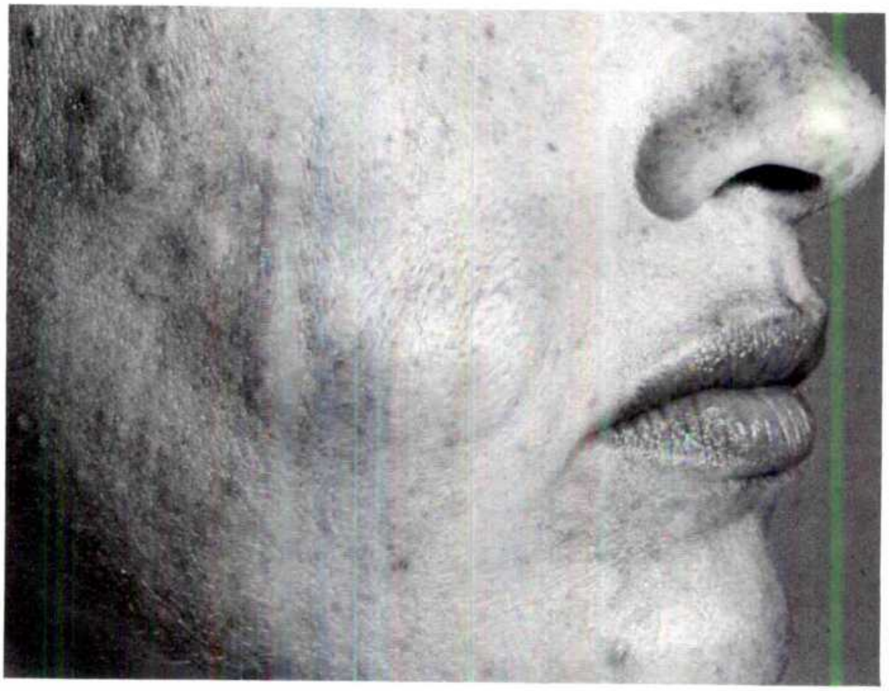

FIGURE 3. Lax, inelastic pouch of skin, a result of long exposure to IR radiation from a hair dryer.

importance. Heat could contribute significantly to UV-induced premature ageing changes, including precancers and cancers. Past studies were crude by today's standards, but the results were sufficiently interesting to have merited greater attention.

In 1943, Bain, Rusch and Klein reported a shortening of the latent period for UV-induced tumours in mice housed at 38°C. Freeman & Knox (1964) found that heat enhanced both the immediate and delayed effects of UV irradiation in haired mice living at 34°C. These animals developed intense erythema, crusting and ulceration while animals living at a lower temperature had only mild erythema with slight crusting. The latency period for UV-induced tumorigenesis was also shorter at the higher temperature.

Recently, it has been shown that heat, in the range of 41–43°C significantly inhibits DNA repair mechanisms (Corry, Robinson & Getz, 1977) and acts synergistically with UV radiation to denature DNA in squamous cells of the buccal mucosa (Roth & London, 1977). The role of IR in cutaneous carcinogenesis is probably significant and deserves far more attention.

Since biochemical reactions are strongly heat dependent, it is entirely possible that hitherto unrecognized chromophores produced by UV radiation are then acted upon by IR to hasten the degenerative and carcinogenic processes. Such a possibility is enhanced by our recent report on the intensification by IR of UV-induced dermal damage in guinea-pigs (Kligman, 1982). Prior to this, there were no experimental studies on the chronic effects of IR on connective tissue. One of the markers of chronic actinic or thermal damage is elastic fibre hyperplasia. The fibres not only proliferate, they become thicker, branched and twisted, resulting in the disordered overgrowth termed elastosis. We have produced extensive elastosis in hairless mice with chronic UV irradiation (Kligman, Akin & Kligman, 1982). Mice, however, develop many skin tumours during the course of irradiation (Winkelmann, Baldes & Zollman, 1960; Epstein & Epstein, 1963; Kligman & Kligman, 1981). We also produced elastic fibre hyperplasia in guinea-pigs without the complication of lethal tumours. We therefore chose this animal in which to investigate the interaction of UV and IR.

Groups of albino guinea-pigs, epilated weekly, were irradiated with either a UV-B light source, an IR source, or the two types of irradiations consecutively, for a total of 45 weeks. With IR, the temperature at the skin's surface was 40°C. In comparison to unirradiated controls, guinea-pigs receiving UV-B or IR alone showed a modest increase in the quantity and thickness of elastic fibres. Additionally, IR alone produced dense skeins of fine, feathery fibres. The combined irradiations, UV followed by IR, produced not only substantially increased amounts of thickened fibres but dense mats resulting from the additional formation of many fine, parallel skeins of elastic fibres.

In contrast to the hairless mouse, guinea-pig collagen proved to be extremely resistant to damage even after prolonged irradiation. As in the mouse, guinea-pig dermis developed increased amounts of ground substance (proteoglycans) with both UV and IR irradiation.

Conclusions

The guinea-pig studies show unequivocally that IR is capable of greatly enhancing elastic fibre proliferation induced by UV irradiation. IR alone evokes significant fibre hyperplasia. This is not a direct effect on dermal fibres, but results from increased synthesis of elastin and microfibrils by irradiated fibroblasts. Adding up the epidemiological and clinical evidence, we believe that IR radiation strongly contributes to chronic sun damage. This includes the well-known stigmata of actinically insulted skin, i.e. mottling, leathery dryness, looseness, wrinkling, loss of elasticity, yellowing and the various actinic tumours. These changes do not occur in protected skin. IR damage is increasingly likely with the greater use of fireplaces, wood stoves, space heaters and various other heat delivering appliances. Finally, wherever insolation is high, heat is a concomitant factor. Sunbathing in midday sun entails being warmed by the far from innocuous IR rays and the perfect sunscreen of the future should contain opalescent materials designed to reflect heat away from skin.

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British Journal of Dermatology (1984) **110**, 376–377.

Obituary

Dr L.J.A.Loewenthal M.D., F.R.C.P. (1903–1983)

Leonard Joseph Alphonse Loewenthal, an Overseas Member of the British Association of Dermatologists, was for 40 years the most distinguished dermatologist on the African continent, and quite possibly the most eminent of Britain's dermatological expatriates in his day. Born in Liverpool, the son of a respected physician, Dr Max Loewenthal, he pursued a brilliant academic career at Malvern College and Liverpool University, from which he qualified in medicine with honours in 1925.

Training in dermatology over the next few years under R.W.MacKenna in Liverpool and P.G.Unna in Hamburg, he obtained at the same time the Liverpool qualifications in tropical medicine and hygiene. He left Britain in 1930 and joined the Colonial Medical Service. Loewenthal's professional career fell thereafter into three phases—a spell of 10 years spent in various parts of Uganda, 5 years in military service in Africa and Italy during the Second World War, and a final 25 years as a dermatological consultant in Johannesburg. In each of these phases his interest and productivity in dermatology were phenomenal.

Despite the multifarious demands of the medical service in Uganda, Loewenthal steadfastly pursued his dermatological investigations. By comparing skin diseases among those of differing dietary habits, he saw the relationship of poor nutrition to tropical ulcer and identified the 'pityriasis-rubra-pilaris-like lesion' of vitamin A deficiency in 1933. Studies on chronic lymphoedema led to an M.D. thesis on 'mossy foot' (1934), and this was followed by a series of articles on skin diseases in the Negro. He was quick to recognize the special place of Kaposi's sarcoma and xeroderma pigmentosum in the African. From Uganda he obtained the London M.R.C.P. in 1934, and was elected to the Fellowship in 1968.

While on military service he continued to publish dermatological articles based on his clinical experiences—on xanthism in Nigerian troops, lichenoid eruptions from antimalarials, and on onchocerciasis. Settling in Johannesburg after the war, he soon transformed the dermatological scene through his lively interest in the local cases and in general questions of dermatological importance. Among a large number of publications, his better-known contributions from Johannesburg were devoted to itching purpura, to the relationships between dermatitis herpetiformis and chronic eczema, and to sweat gland function in workers on the gold mines. Of his contributions to books, one may mention the chapters in Mitchell Heggs' *Modern Practice in Dermatology* (1950), *The Eczemas* published by Livingstone (1954), a booklet on Yaws published with C.J.Hackett (1960), and a large section on 'Ethnology in Dermatology' for Jadassohn's *Handbuch* (1967).

After retirement he settled in Spain, near Barcelona, where he wrote five remarkable books devoted to Spain and Italy. These were: *The Catalan Vengeance* (London: Routledge, 1972), *The Barrier and the Bridge* (London: Geoffrey Bles, 1972), *Companion Guide to Southern Spain* (London: Collins, 1973), *La Serenissima. The Last Flowering of the Venetian Republic* (London: Cassell, 1974), and *Culture and History of the Spanish* (London: Gordon and Cremonesi, 1977). In these works he dropped his medical image and published under the name of 'Alfonso Lowe, Correspondent of the Royal Academy of Córdoba'. After the death of his wife in Spain, Loewenthal returned to Africa, and died in Cape Town in March 1983.

In company, Loewenthal was noted for his immensely lively wit, swift intelligence and great cheerfulness. Behind this public image he lived the life of a scholarly recluse, building up the stores of knowledge which he used to such advantage.

One of his greatest gifts lay in a beautiful command of language, as can be seen in his later writings. Of his prose Cyril Connolly said: 'How good is the moment when one discovers that the author can write, and that one is really going to learn something'. Another great quality was his eager application and forward-looking attitude to 'continuing education'.

For sustained productivity, there cannot be many others who wrote over 200 papers, and turned out books and monographs in the half-century from 1930 onwards as he did, particularly if one takes the circumstances into account. His academic connections and contact with colleagues of eminence were often quite tenuous. To his unaided initiative must be given the greatest credit for what he achieved, without the comfort and shelter which most people find necessary to accomplish what he did purely on his own. Many were deceived by the lightness with which he carried his knowledge, and underestimated the effort needed to reach his level of eminence. A rare and lasting stamina made his achievements possible in circumstances which would have destroyed the enthusiasm of many delicate spirits. He bore frustration without regret and had the inner confidence to make the best of each new situation. Taken all round, Loewenthal loved both his life and his work in dermatology.

G.H.FINDLAY

British Journal of Dermatology (1984) **110**, 378–380

Correspondence

Bullous pemphigoid resembling erythema annulare centrifugum

SIR, Blisters in bullous pemphigoid commonly occur on an erythematous base which may spread, becoming confluent. We have recently seen a patient whose bullous pemphigoid resembled erythema annulare centrifugum. This is a rare occurrence but has been reported previously (Vakilzadeh & Suter, 1981), and Post (1984) has recently reported a patient with pemphigus presenting as erythema annulare centrifugum.

Our patient, a 60-year-old man, presented with a 3-month history of a pruritic rash which began in the umbilicus, becoming widespread with discrete lesions on the trunk and limbs. His rash consisted of circular erythematous lesions 2 to 5 cm in diameter with a raised, palpable edge. There were tense 1 to 2 cm blisters within the larger lesions. Two skin biopsies showed subepidermal bullae in which inflammatory cells were prominent, with aggregation of polymorphs and eosinophils in the dermal papillae, and a perivascular chronic inflammatory cell infiltrate in the dermis. Direct immunofluorescence showed linear deposition of C3 along the basement membrane zone in titres up to 1:640. Other routine investigations revealed no other abnormalities.

Treatment was commenced with prednisolone 60 mg daily, later increased to 70 mg daily. After 2 weeks azathioprine was added in a dose of 150 mg daily. A trial of dapsone therapy in a dose of 50 mg daily for 2

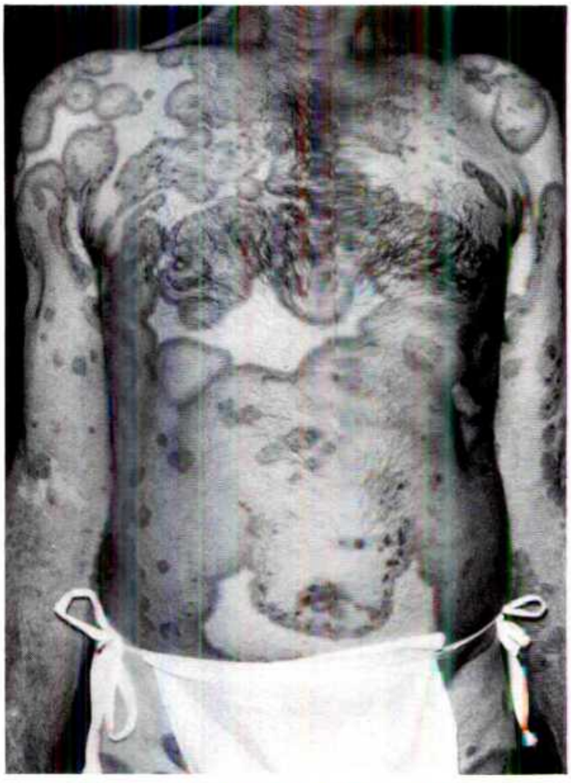

FIGURE 1. The rash 1 month after admission showing centrifugally expanding erythema with blistering in the active margin.

weeks was without effect. Over the next 2 months the rash slowly extended centrifugally becoming confluent with pigmentation centrally and blistering occurring along the advancing edge (Fig. 1). After 3 months the rash was controlled and 1 year later prednisolone was reduced to 15 mg daily without relapse.

Reports of this appearance in bullous pemphigoid are rare (Vakilzadeh & Suter, 1981) but there are some similarities with the bullous variant of erythema annulare centrifugum described by Kind (1975). Other patients with bullous pemphigoid have had transient erythema gyratum repens-like rashes (Breathnach, Wilkinson & Black, 1982; Saikia, Mackie & McQueen, 1973). IgG and C3 immunofluorescence have been seen along the basement membrane zone in erythema annulare centrifugum (Holt & Davies, 1976). Perhaps there is some similar mechanism involved in the pathogenesis of annular erythemas and these cases of bullous pemphigoid, particularly with regard to the spread of the rash from involved to uninvolved skin, which might depend on coupled immune interactions linked by diffusion or migratory immunoregulatory factors as postulated by Moore (1982).

ACKNOWLEDGMENT

We wish to thank Dr S.M.Hadi, Department of Dermatology, The Royal Infirmary, Edinburgh for the indirect immunofluorescence titres done on this patient.

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Natural killer cells involved in lymphomatoid granulomatosis

Sir, We were most interested to read the comment of Rooney *et al.* (*British Journal of Dermatology* 1984, **110,** 248) on our case of lymphomatoid granulomatosis (Bos *et al.*, 1983). Although we made no mention of it, the granulomas observed were indeed angiocentric and angiodestructive. The combination of central nervous system involvement, pulmonary involvement and widespread skin lesions with the histopathological picture of lymphomatoid granulomatosis, seems to fulfill all the criteria for the diagnosis of lymphomatoid granulomatosis (Liebow, Carrington & Friedman, 1972). We ended our report with the suggestion that our case might be developing into a high grade malignant non-Hodgkin lymphoma.

At present we do not feel it is appropriate for immunophenotyping by monoclonal antibodies to be necessary for the diagnosis. Immunophenotyping by this new technique is still in its infancy. In view of the fact that so few cases of lymphomatoid granulomatosis have been studied by this new approach, we believe it is more realistic to assume that lymphomatoid granulomatosis may not always be an immunopathological entity (see also Bender & Jaffe, 1980). It may be subdivided into cases such as the patient described by Harrington *et al.* (1983), where T_4^+ and T[illegible]$^+$ lymphocytes are the principal cells involved, and into cases such as ours (Bos *et al.*, 1983), in which the principal cells are histiocytic (and as yet not accessible for monoclonal antibody studies) accompanied by an extremely uncommon and relatively high percentage of HNK-1$^+$ natural killer cells.

In view of the relative rarity of the syndrome, we hope that as many cases as possible will be studied by immunophenotyping to clarify the issue.

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British Journal of Dermatology (1983) **110,** 381–383.

Book Reviews

Cutaneous Infestations of Man and Animal. Edited by L.C.PARISH, W.B.NUTTING and R.M.SCHWARTZMAN (1983) New York: Praeger. Pp. 392. Price £39.50.

In 1980 a conference was held in Philadelphia on cutaneous infestations of man and other animals. The present volume is an embellished and expanded version of the proceedings of that conference. The result is an exceedingly useful book. Its scope is wider than its title indicates, for it includes in addition to the infestations other types of skin lesions caused by arthropods, and also devotes a chapter to non-arthropod parasites such as leishmaniasis and onchocerciasis. It would perhaps have been better had these non-arthropod parasites been omitted, for the chapter does not do justice to their great importance in many parts of the world, and additional space could usefully have been employed to expand the accounts of some of the arthropods.

Most of the authors have provided good lists of references to the international literature; in fact although the relevant literature is very widely scattered those working in this field are well served by the excellent abstract journals published by the Commonwealth Bureau of Entomology. The book appropriately devotes most space to scabies and pediculosis and reminds us there are still vast gaps in our knowledge of these common diseases.

ARTHUR ROOK

Skin. By P.F.MILLINGTON and R.WILKINSON (1983) Cambridge: Cambridge University Press. Pp. 224. Price £35.00.

I suppose that even the least sophisticated of us would not expect to find the whole of skin biology in a book of 224 pages, even though it was just entitled 'Skin'. Nonetheless I think the authors could have found a more imaginative title for this interesting but patchy monograph. Essentially it deals with the skin as a physical structure, and it is to be commended as one of the few books that have attempted to cover this particular subject. Of most value to your reviewer is a chapter on the nature of the skin surface, its various markings and site variations. The sections on the appendages and on the overall structure are not as good. Certainly the average dermatologist-in-training will require more information, and I fear there are some inaccuracies. I was also disturbed to see a sketchy section on epidermal growth and larger sections on the epidermal proliferating unit and chalones, these latter two subjects causing heated debate amongst some skin biologists.

The sections on the mechanical properties and electrical properties are good, although it is a shame that when reference to disease is made there are once again inaccuracies, and inadequacies, in the text. There are also chapters on the effects of environmental agents on the skin and on wound closure and alternatives to skin which could be useful to dermatologists in training.

All in all, this is an interesting little book which is quite well illustrated. However, it is in places guilty of the sins of both omission and commission, and I trust that future editions will improve.

R.MARKS

Contact and Photocontact Allergens, a Manual of Predictive Test Methods. By THOMAS MAURER (1983) New York: Marcel Dekker. Pp. 179. Price S Fr 79.00.

The testing of chemicals for their contact allergic and photocontact allergic potential in the guinea-pig has become an essential part of preventive dermatology. There is probably no substitute for learning such techniques at the side of someone already proficient in them, but there is a need for a manual to consult while starting out on such training. What is more, dermatologists, toxicologists and occupational physicians all require some understanding of such techniques to help them to assess test results for chemicals on which they are asked for advice. It is for all such professionals that this latest edition to an excellent new series on dermatology will be invaluable.

It is a practical and up-to-date review of the field and it has the great advantage of being written by a foremost exponent of guinea-pig testing, Dr Thomas Maurer. The selection of tests discussed has been made on the basis of his own lengthy personal experience, the mass of published data available and his knowledge of which tests are routinely used in laboratories worldwide. It is an extremely well-referenced book and many of these references date from the last 2 years. The writing is clear and understandable.

The essential three chapters in the book are those on general considerations in guinea-pig testing, guinea-pig methods for assessing contact allergic potential and guinea-pig methods for assessing photocontact allergic potential. Methods that are considered less good by the author are described, as well as those that he prefers, and reasons are clearly given for his preference. In each case the predictability provided by results is discussed. Details of techniques are helpfully included.

As background to these three chapters there are brief reviews of the immunology and epidemiology of allergic and photoallergic contact dermatitis. The book makes no attempt to review immunological mechanisms in detail or to cover the *in vitro* testing of chemicals. This is a book for the physician and toxicologist rather than the immunologist. I have no hesitation in recommending it as essential reading for any dermatologist who wishes to familiarize himself with these extremely valuable techniques.

R J.G.RYCROFT

Technical Guide for Smear Examination for Leprosy by Direct Microscopy. By D.L.LEIKER and A.C.MCDOUGALL (1983) Amsterdam: Leprosy Documentation Service. Pp 34. Price approx. 50 pence.

The authors of this booklet are both very experienced leprologists and as a result it gives an excellent account of the techniques involved in skin smear examination in leprosy. It goes into great detail, describing the sites of selection, the usefulness of nasal smears, the ingredients of the appropriate stains and most importantly, the interpretation of the tests. It has been produced partly as a result of a declaration by the WHO expert committee on leprosy which expressed concern at the extremely low standard of the bacteriological examination techniques used in many leprosy control projects, and stressed the need to improve them.

It is worth recording that there is commonly a misconception about the usefulness of smear examination. In general it is more useful as a test of prognosis rather than of diagnosis. Usually bacilli are seen only in patients with borderline lepromatous and polar lepromatous leprosy, where there should be little difficulty in making the diagnosis clinically, though in some patients with borderline leprosy, the diffuse hypopigmentation may be difficult to define, and in diffuse lepromatous leprosy the diagnosis may be difficult. In these patients the skin smear is a useful diagnostic test. However, it is much more useful as a prognostic test, especially now that dapsone resistance is rife throughout the tropics. The examination of skin smears sequentially at 6-monthly or yearly intervals may reveal that the bacterial index is not falling as expected, or that it falls initially and then rises again. Also the morphological index may continue to be positive. This indicates that the patient may not be taking his drugs, or more importantly may be the first indication of drug resistance, in which case other drugs must be used before further nerve damage occurs.

For those who manage leprosy patients, this is an extremely useful little booklet, and I thoroughly recommend it.

R.STC.BARNETSON

Cutaneous Laser Therapy: Principles and Methods. Edited by K.A.Arndt, J.M.Noe and S.Rosen (1983) Chichester: John Wiley. Pp. 241. Price £25.00.

The rapidly increasing interest in cutaneous laser therapy will be stimulated by the writings of the seventeen contributors to this book. They combine to produce a practical guide which is essential reading for dermatologists, plastic surgeons and others involved in treating haemangiomas, tattoos, tumours, etc. Lasers of various types are coming to play an increasingly important role in the management of some difficult problems and those involved should be aware of the current practicalities and the future possibilities.

I enjoyed reading the book but at the end I was left with a feeling of disappointment which it is hard to explain. Firmer editorial control could have prevented some of the feeling of *déjà vu* almost inevitable in a symposium. For a book in which one-third of the content is devoted to 'port wine stains' I was amazed to find no colour photographs. Whether argon laser therapy will stand alone, be an essential evolutionary step or pass into the mists to join Thorium X, will depend on the long-term results. These cannot be evaluated from black and white photographs, especially when the post-treatment picture is obviously over-exposed. At times the book is either misleading or frankly incorrect. Coherence is badly defined and surely a cutaneous haemangioma is an essential part of the Sturge–Weber syndrome. The number of typographical errors cannot be excused.

Despite early enthusiasm dermatology has lagged behind other disciplines in using lasers therapeutically. This book gives the reader a valuable insight which could encourage an expansion of facilities and much needed research.

Neil Walker

Book received: *Incidence of Nonmelanoma Skin Cancer in the United States.* By J.Scotto, T.R.Fears, and J.F.Fraumeni, Jr. NIH Publ. No. 83–2433, Washington, D.C., U.S. Govt. Printing Office, 1983.

British Journal of Dermatology (1984) **110**, 384.

News and Notices

Annual Meeting of British Society for Investigative Dermatology, Keele, 1984

The next annual meeting of the B.S.I.D. will take place on 20–21 September 1984, in Keele University. The scientific programme will include free communications (oral and poster), guest lectures and workshops. The meeting is open to non-members of the Society. Abstracts should be submitted on the official forms and must be received by Dr R.A.J.Eady by 8 June 1984. For further information and abstract forms, please write to: Dr R.A.J.Eady, Chairman, B.S.I.D., Institute of Dermatology, Homerton Grove, London E9 6BX, U.K.

Third International Symposium of Dermatopathology, Liège, August 1984

This symposium, entitled 'Malignant melanomas and other neurocristopathies' will be held in Liège, Belgium, between 11 and 15 August 1984. A distinguished faculty of thirty-eight dermatologists and pathologists has been invited to present brief didactic presentations followed by panel discussions in which subtleties of differential diagnosis will be explored. The symposium will take place in the Convention Centre of Liège and accommodation is being reserved. Further informations may be obtained from: G.Pierard, Department of Dermatology, Hôpital de Bavière, Bd. de la Constitution, 66, B-4020 Liège, Belgium, or A.B.Ackerman, Department of Dermatology, New York University School of Medicine, New York, NY 10016, U.S.A.

Fifth International Colloquium of Dermatopathology, Liège, August 1984

This colloquium of the International Society of Dermatopathology will be held on Friday, 10 August 1984, immediately preceding the Third International Dermatopathology symposium in Liège, Belgium. Abstracts (no more than 250 words) should be submitted before 1 March 1984 to: C. Franchimont, Department of Dermatology, CHU de Bavière, Bd. de la Constitution 66, B-4020 Liège, Belgium. The meeting will take place in the Convention Centre of Liège, and accommodation will be reserved in hotels. The registration fee, including lunch, is 2,000 Belgian francs.

Skin Pharmacology Society

Following an informal gathering of dermatologists and pharmacologists in London in September 1983 a new research group has been formed, entitled the Skin Pharmacology Society. The aims of the Society, which is internationally based, are: (i) to promote and encourage pharmacological research in the skin (including skin toxicology); (ii) to introduce pharmacological knowledge and expertise into dermatological research; (ii) to bring recent advances in skin pharmacology to the attention of general pharmacologists; (iv) to encourage interest in the pathophysiology of the skin amongst general pharmacologists; (v) to enhance standards of pharmacological research in the skin; (vi) to organize at least one scientific meeting annually; (vii) to foster exchange of ideas with pharmacological colleagues in industry.

The Society will hold its first scientific meeting as a satellite symposium of the 1984 IUPHAR 9th International Congress of Pharmacology, London. The title of the symposium is 'Mediators of inflammatory and proliferative skin disorders' and it will be held in the Barbican Centre, London, 4–5 August 1984. Further details of the Skin Pharmacology Society and its first symposium can be obtained from M.W.Greaves (Chairman), Institute of Dermatology, Homerton Grove, Hackney, London E9 6BX, England.

Watson Smith Lecture

The Watson Smith Lecture at the Royal College of Physicians of London will be delivered by Dr N.R. Rowell, on Wednesday April 11, 1984 at 6 p.m. The subject is systemic sclerosis.

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Pediatric Dermatology

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Pediatric Dermatology is a new international journal which is closely associated with the Society for Pediatric Dermatology and the International Society of Pediatric Dermatology. Its primary purpose is to publish original papers relating to clinical and laboratory investigations, case reports, review articles relevant to the speciality of pediatric dermatology, editorial comments and book reviews.

Pediatric Dermatology aims to further the knowledge of both pediatricians and dermatologists regarding skin diseases of infants, children and adolescents. It particularly supports the contention that skin diseases are ultimately related to the workings of the organism as a whole and may reflect physiological as well as psychological alterations. The journal therefore welcomes contributions which are critical, authoritative and directly related to the structure, function and disease of the skin. These studies may be biochemical, pharmacological, histological, immunological, electron microscopic, or clinical in nature.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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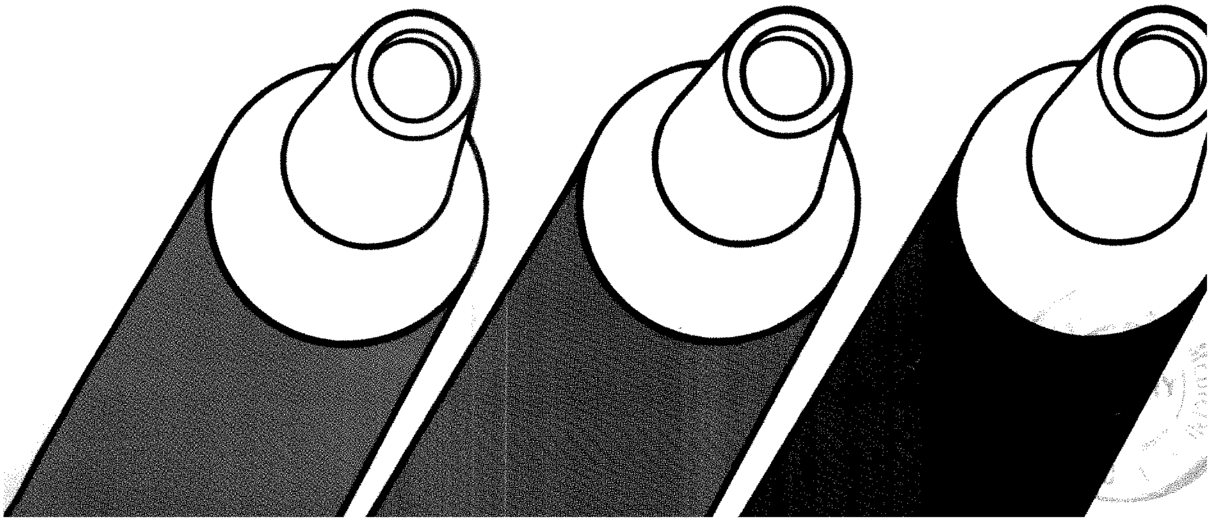

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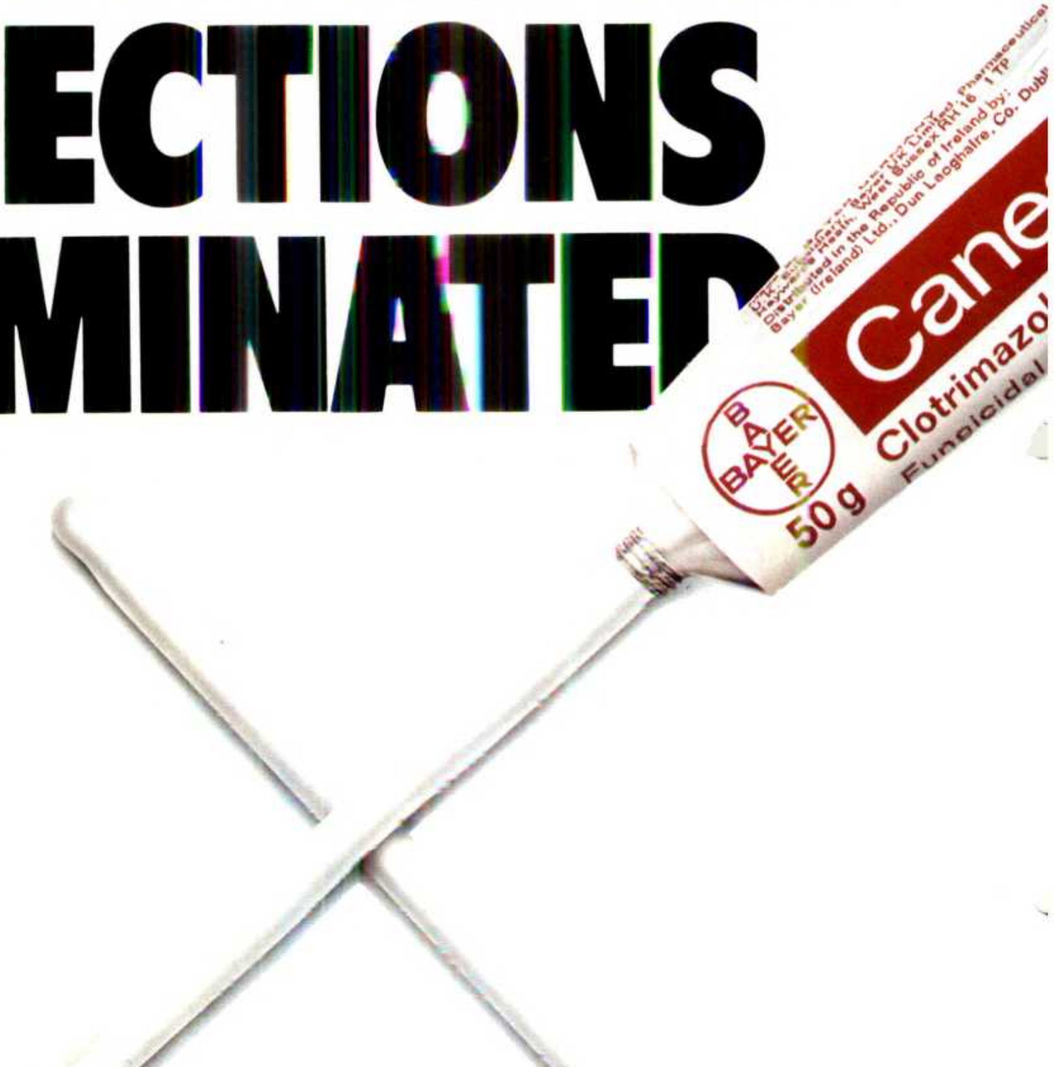
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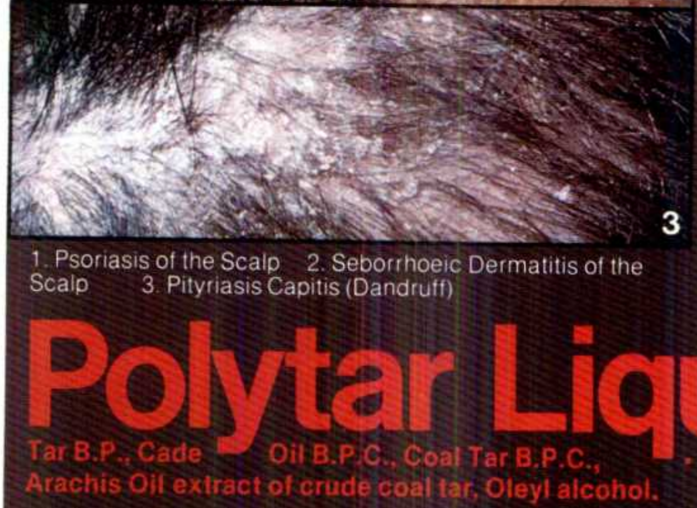

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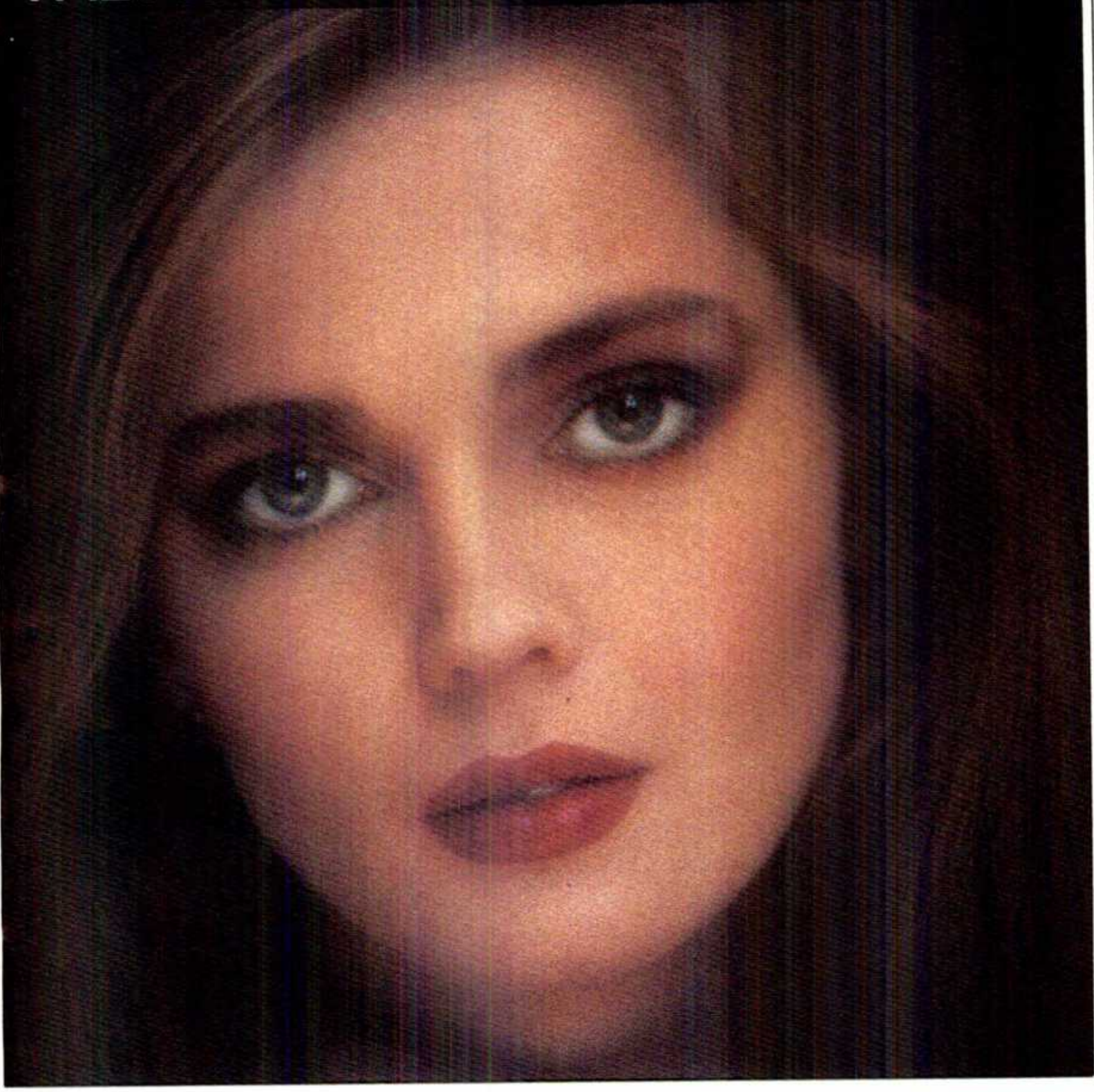

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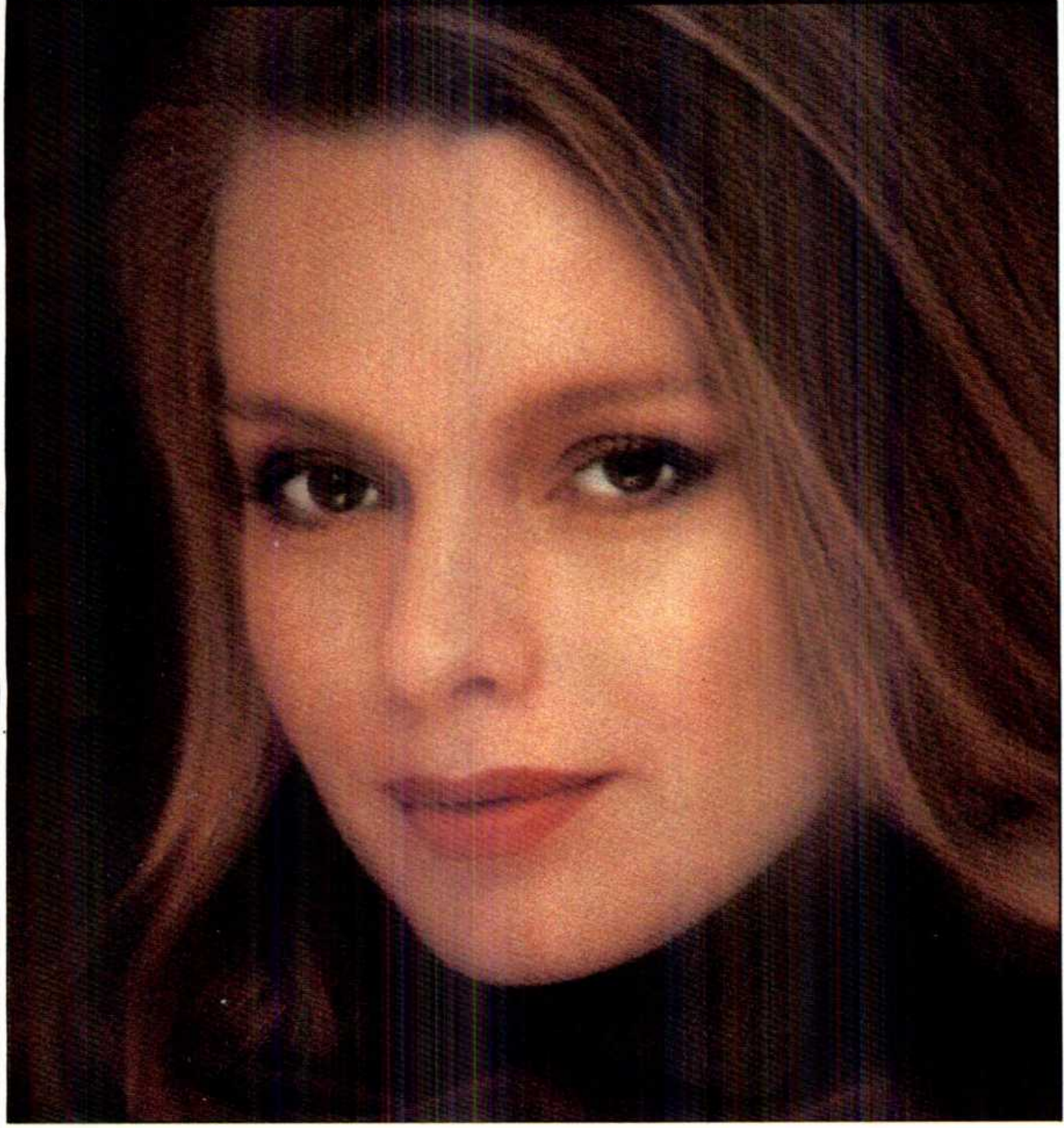

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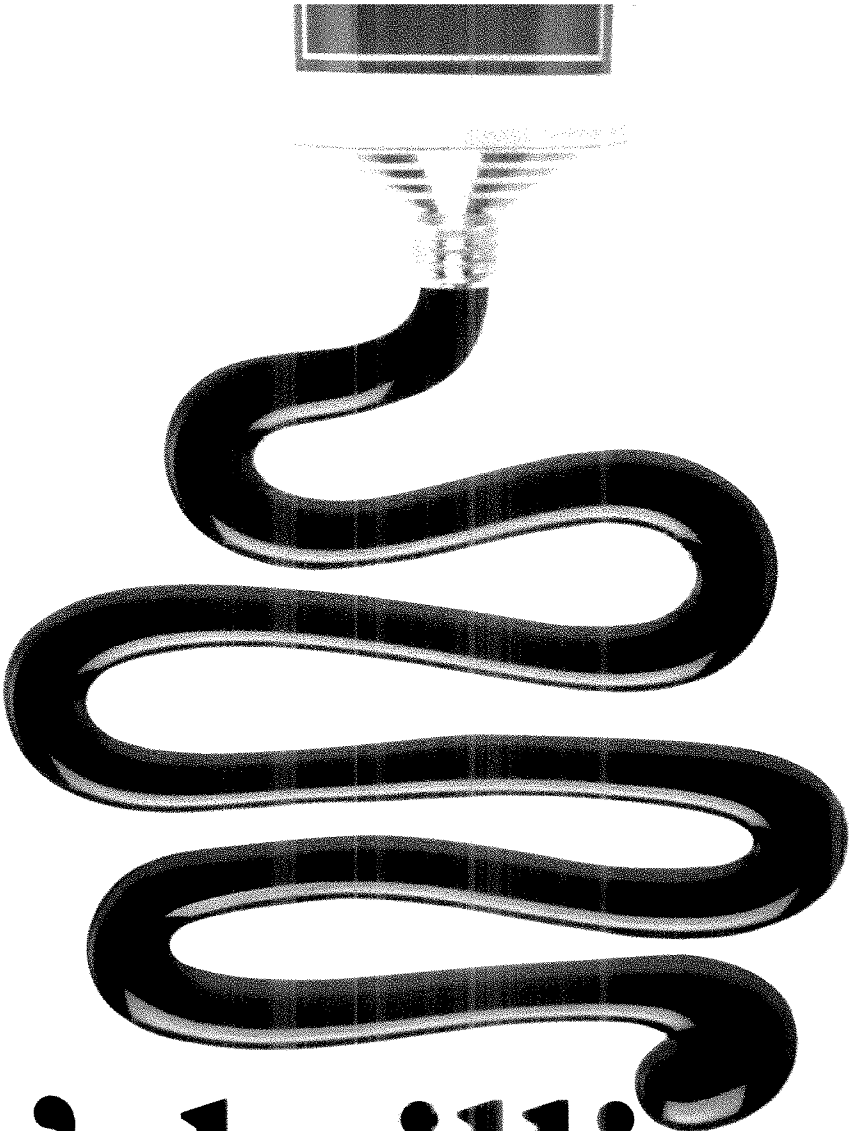

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Clinical and Laboratory Investigations

The effects of thermal stimulation on the ultrastructure of the human atrichial sweat gland. I. The fundus

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SUMMARY

Ultrastructural examination of sweat glands from the human loin before and during heat-induced activity indicated that the sweat is formed from the contents of disrupted cells as well as from the products of secretion. The principal secretory processes appear to be fluid transport and exocytosis of vesicles. However, configurations suggesting microapocrine secretion were also observed. It is concluded that the mechanisms involved in sweat production in man are fundamentally similar to those in animals and the terms 'apocrine' and 'eccrine' should be discarded. The myoepithelial cells which were contracted at the onset of sweating appeared to be under less tension after 3 h of continuous activity.

The sweat glands of haired animals and specific hairy body regions of man, which develop from the hair anlage and are closely associated with hair follicles (i.e. epitrichial) have long been classed as 'apocrine' (Schiefferdecker, 1917). However, ultrastructural studies of the epitrichial glands of the human axilla after adrenaline injection (Bell, 1974) and of domestic animals during thermal stimulation (Jenkinson, Montgomery & Elder, 1979; Montgomery, Jenkinson & Elder, 1982a, b) reveal a basic mode of sweat formation quite different from the classical concept of apocrine secretion. These studies of glands at different stages of secretion indicate that the sweat is composed of products which arise (1) from secretory processes, mainly fluid transport and exocytosis of vesicles, although a secondary mechanism of vesicle loss by microapocrine secretion may occur in some instances, and (2) from cells which can no longer meet the demands of stimulation and are shed from the secretory epithelium. The main function of the outer myoepithelial layer of the fundus which seems to remain contracted throughout sweating may, as suggested by Sato, Nishiyama & Kobayashi (1979) and Sato (1980), be the provision of structural support to the secretory epithelium. The ducts of the epitrichial sweat glands, although likely to be involved in ion reabsorption, exhibit little morphological change during activity other than distension of the lumen and intercellular spaces.

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The sweat glands of the body surface of some primates, including man, and of the footpads of the dog and cat, which arise from the epidermis and are not associated with hair follicles (i.e. atrichial) were termed 'eccrine' by Schiefferdecker (1917) who considered them to differ physiologically from the epitrichial glands. This traditional concept and classification, although challenged (Dobson *et al.*, 1958; Jenkinson, 1967, 1973) has been retained.

However, histochemical and light microscopical studies of the human atrichial gland (Dobson *et al.*, 1958) illustrate that, during activity, some cells vacuolate and retain glycogen, while others exhibit particle and glycogen depletion. The vacuolated cells are apparently damaged to the extent that they are unable to function normally. Thus, although the authors at that time considered it unlikely that such a normal function as secretion of sweat would produce 'frank damage' to the secretory cells, it now seems quite possible, in the light of the above evidence on active epitrichial glands, that cell disruption and death may also occur in atrichial glands during secretion.

It is now also conceivable that these glands do not differ appreciably from epitrichial glands in their mode of secretion; information on their ultrastructure appears to be limited to the unstimulated state (Ellis, 1962, 1965; Montagna & Parakkal, 1974; Sato, 1977; Hashimoto, 1978, Kurosumi, Kurosumi & Tosaka, 1982) apart from a recent study of the distribution of calcium pyroantimonate complexes in the glands of the rat footpad (Sampson & Bowers, 1982). The concept of physiologically distinct sweat gland types therefore, requires reappraisal after more detailed scrutiny of the active atrichial gland. Consequently, the ultrastructure of the human atrichial gland was examined before and during thermally induced activity and compared with equivalent data on epitrichial sweat glands as a prelude to projected comparative studies by electron probe microanalysis of the ionic changes in the glands during secretion.

METHODS

Procedure

Four men, aged 20–22 years, kept previously for 1 h in an ambient environment of 16 °C Dry Bulb (DB)/9 °C Wet Bulb (WB), were exposed to 40 °C DB/30 °C WB in a climatic chamber for up to 4 h. Cutaneous moisture loss was measured continuously throughout this period from an area on the loin using the ventilated capsule technique of McLean (1963). A skin sample, 3 mm in diameter, was removed by biopsy (immediately after administration of 0·1 ml of 2% xylocaine) from the contralateral area on each individual before, at the onset of, and during sweating, as shown in Fig. 1.

Histology

All skin specimens were immediately cut into several pieces, fixed for 3 h in 2% glutaraldehyde in 0·1 M sodium cacodylate buffer at pH 7·3 and processed for electron microscopy, as described by Montgomery *et al.* (1982b). Three or four glands within every skin sample were serially sectioned at 1 μm parallel to the skin surface. Ultrathin sections were also cut during this process, at 20 μm intervals throughout the intraepithelial zone and at 50 μm intervals along the duct body and fundus. The 1 μm sections were examined by light microscopy after staining with toluidine blue/pyronin Y (Ito & Winchester, 1963). Ultrathin specimens cut perpendicular to the skin surface were also studied.

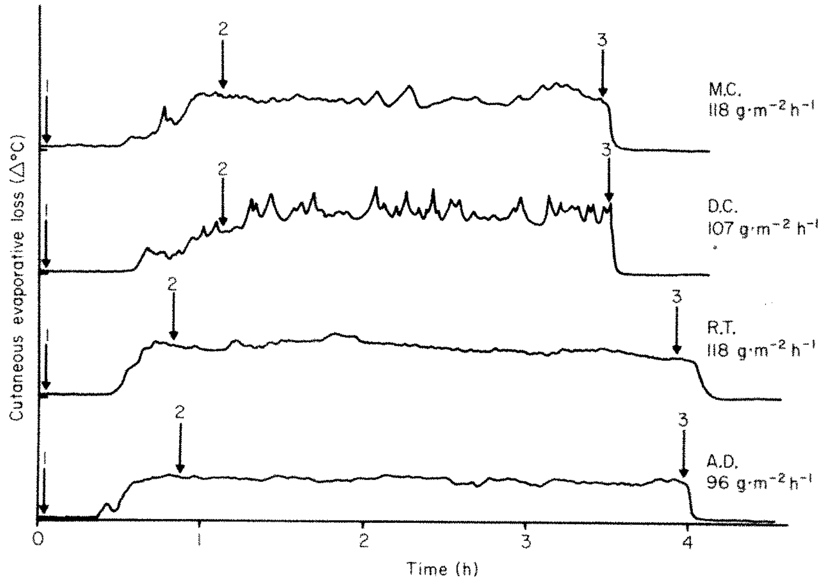

FIGURE 1. The patterns of evaporative loss obtained from four human subjects upon exposure to the warm environment. Skin samples were taken from the contralateral side at the times shown (arrows). Mean determinations of the rate of sweat output are given in each instance.

RESULTS

Patterns of evaporative loss

The patterns of evaporative loss, each to its own baseline, obtained from the four human males upon subjection to heat are illustrated in Fig. 1. The patterns obtained were comparable, although the magnitude of the response varied between subjects. The output achieved shortly after onset was maintained throughout the period of study.

Unstimulated fundus

The fundus was composed of two cell layers surrounded by connective tissue enclosed in a fenestrated fibrocyte sheath (Fig. 2a and insert). The outer myoepithelial cells were elongated with a convex luminal surface and a generally flattened, although crenated, base attached by hemidesmosomes to a well-developed basal lamina (Fig. 2a). They formed an incomplete layer around the inner secretory epithelium giving the impression of a wide-meshed myoepithelial network around the secretory cells. Caveolae were more prominent along the basal portions of the myoepithelial cells, which contained dense bodies with a degree of alignment giving, in some planes of section, a pseudo-striated appearance to these smooth muscles.

The inner secretory epithelium was composed of two morphologically distinct types of secretory cell (granular and non-granular) which were interdigitated in a manner which often gave the impression of a pseudo-stratified epithelium. Luminal microvilli were sparse, small and stubby in both cell types. The most prominent feature of the more numerous non-granular cells was their complex basal infoldings, fingers of which were also found between the myoepithelium (Fig. 2b) attached to the basal lamina. Mitochondria were numerous adjacent to these and the lateral interdigitations. In some instances adjoining non-granular cells formed canaliculi which were open to the luminal surface and terminated among the basal infoldings (Figs 2a, 3b). The

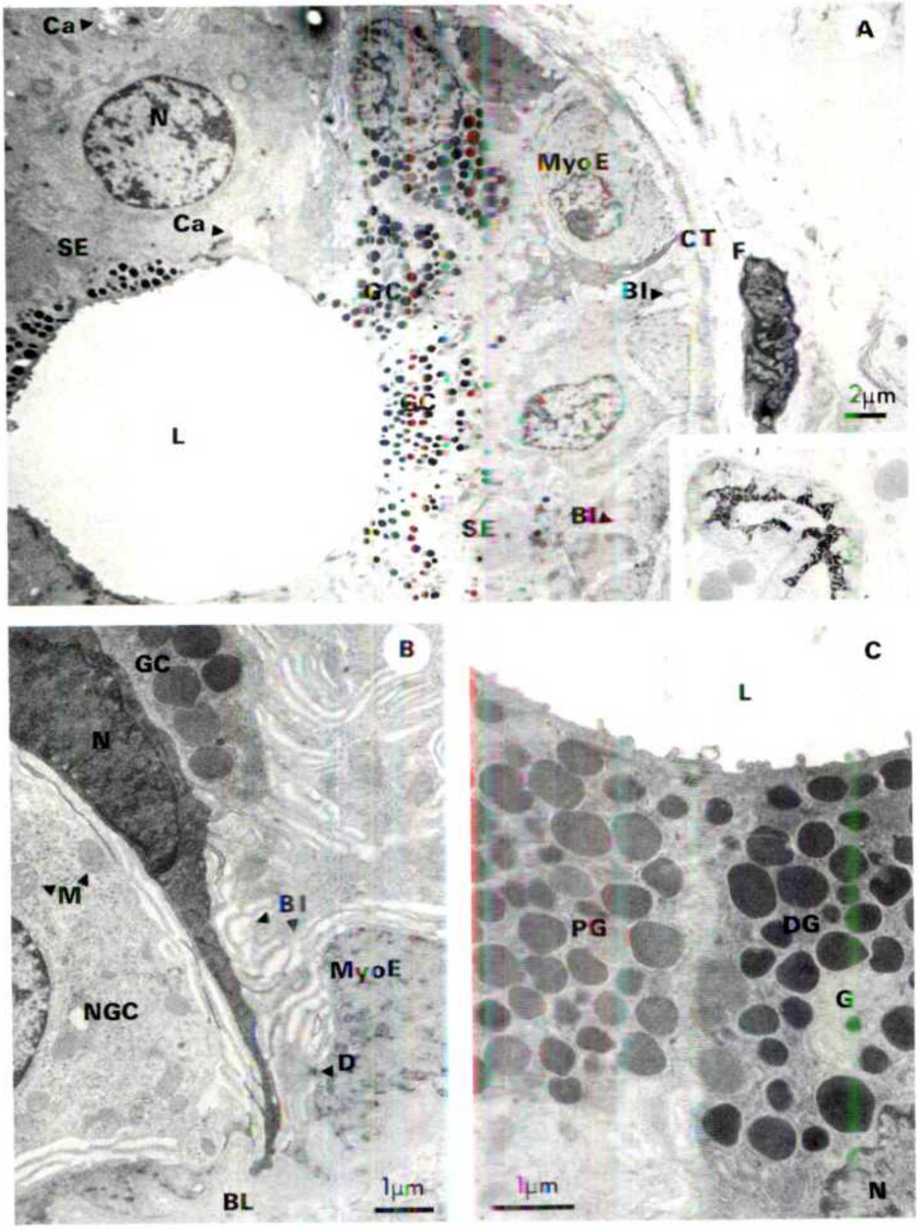

FIGURE 2. (A) Light (insert) and electron micrographs of the fundus of the unstimulated atrichial sweat gland illustrating the secretory and myoepithelial layers and the surrounding connective tissue and fibrocyte sheath. (Lettering on this and subsequent figures according to Key.) The goblet-shaped granular cells are prominent and at higher power the dome-like profiles of the myoepithelial cells and basal infoldings of the non-granular secretory cells extending to the basal lamina are evident. Sections of canaliculi are also visible. (B) High-power view of the base of the unstimulated gland showing the complex basal infoldings of the non-granular cells and a finger of a granular cell extending to the basal lamina. A desmosome connection between a myoepithelial cell and a non-granular cell process can be seen. (C) The granules in some of the granular cells were mostly electron opaque, whereas those which predominated in other cells were visibly paler.

lateral and basal surfaces of these channels were sealed by junctional complexes, including *zonulae occludentes*, between the cells, so that in effect they could be considered as extensions of the luminal membrane. Such canaliculi were not found between granular and non-granular cells or between adjacent granular cells. In both these latter loci, however, neighbouring cells were also joined at the luminal surface by junctional complexes and interconnected laterally by desmosomes and gap junctions. Myoepithelial cells were also connected to interposed projections of adjacent secretory cells by desmosomes (Fig. 2b) and occasional gap junctions. However, desmosome connections between neighbouring myoepithelial cells were rarely seen.

The nuclei of the wine-glass-shaped granular cells were generally situated towards the base of the bowl. However, the stem was only occasionally traced in favourable planes of section to the basal lamina (Fig. 2b). The granules were distributed throughout the cytoplasm but occurred in greater density in the luminal segment of the bowl. A limiting membrane around the granule was generally not easily discerned, but was detected in some instances. The granules exhibited differences in the texture of their contents and those in some cells appeared much darker. Such cells with a predominance of dark granules could be distinguished from others in which the majority of the granules were much lighter (Fig. 2c).

Effects of stimulation

Onset of sweating. The fundus of the active gland differed in appearance from the control gland even when examined by light microscopy. Shortly after the onset of sweating there was an apparent thickening of the basal lamina and vacuoles were observed within the secretory epithelium (insert, Fig. 3a): this was usually associated with a decrease in the size of the lumen.

Electron microscopy illustrated that the smaller lumen at onset contained a fine filamentous material and cell debris (Fig. 3a). The myoepithelial cells were taller and, judging by the puckering of their serosal surfaces, appeared to be under tension. A prominent feature of the secretory epithelium was dilatation of the intercellular spaces and in particular the basolateral infoldings and canaliculi between non-granular cells (Fig. 3a). The basal infoldings were generally (Fig. 3c), although not always (Fig. 3b), markedly distended. The cytoplasm of the non-granular cells, which were now more cuboidal, contained less glycogen and had a foamy appearance as a result of distension of the SER. Cytoplasmic vesiculation was particularly pronounced towards the lumen and adjacent to canaliculi. The matrices of the mitochondria exhibited translucent zones. However, some non-granular cells within the secretory epithelium, identified by their characteristic mitochondria and their proximity to canaliculi, appeared to have accumulated large deposits of glycogen in their cytoplasm (Fig. 4b). Cells with this

Key to Figs 2–5

BI	Basal infoldings
BL	Basal lamina
Ca	Canaliculus
CP	Cytoplasmic protrusions
CR	Cell remnants
CT	Connective tissue
D	Desmosome
DG	Dark (electron opaque) Granules
DM	Disrupted membrane
F	Fibrocyte sheath
G	Golgi apparatus
GC	Granular cell
Gly	Glycogen
L	Lumen
M	Mitochondrion
N	Nucleus
M	Possible microapocrine configuration
MyoE	Myoepithelial cell
NGC	Non-granular cell
PG	Paler granules
SE	Secretory epithelium
V	Vesicles

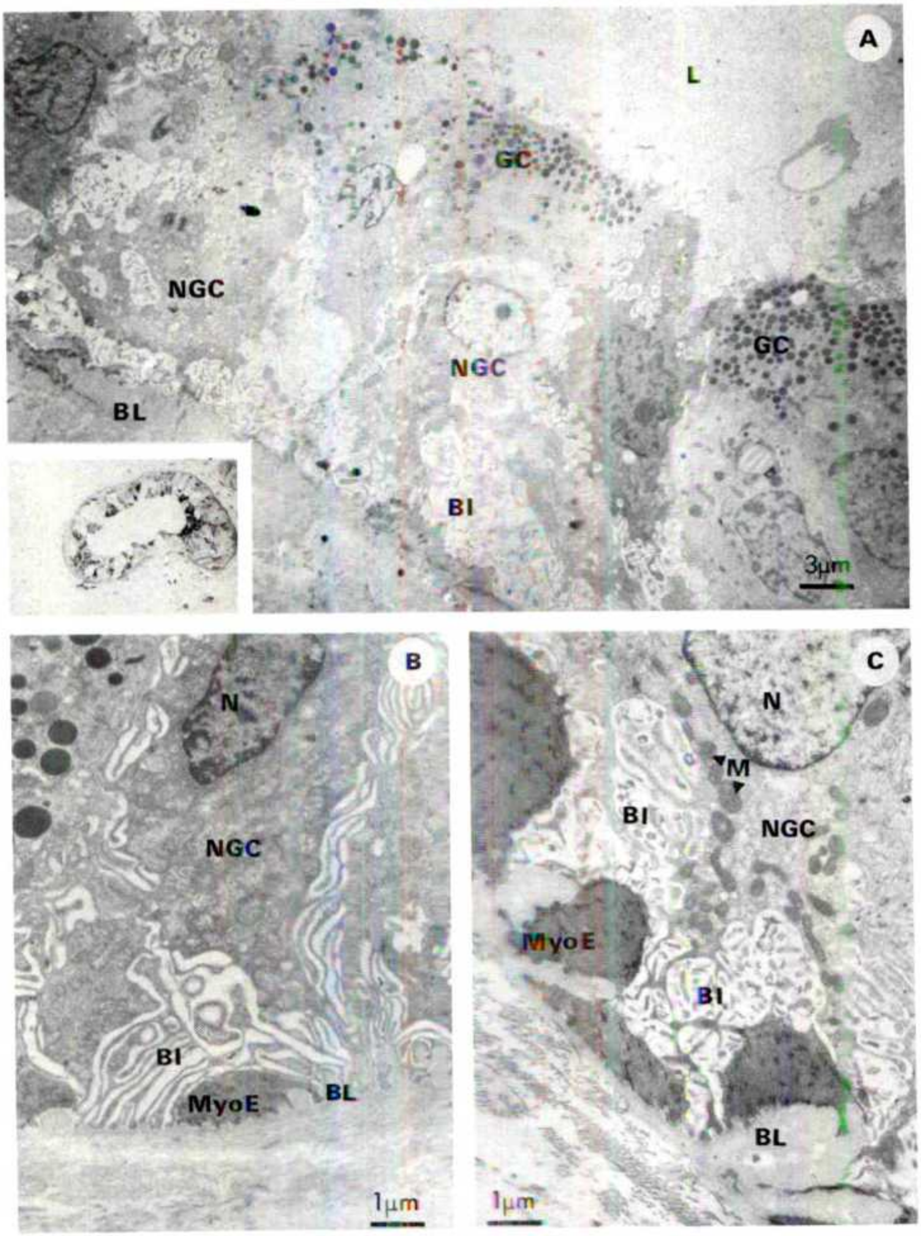

FIGURE 3. (A) The appearance of the fundus at the onset of sweating. At low power (insert) there is evidence of vacuolization of the secretory epithelium. Some granular cells exhibit evidence of granule depletion and the dilatation of the basal infoldings between non-granular cells is striking. Cellular debris is present in the lumen. (B) A high-power view of a section of the base of the fundus at the onset of sweating. In this instance the basal infoldings, although wider than in control sections, are not greatly distended. The basal lamina is prominent. (C) This portion of the basal epithelium at onset illustrates marked dilatation of the basal infoldings and the presence of mitochondria in close proximity to the projections. Comparison with Fig. 3B indicates that the response to stimulation of the non-granular cells like that of the granular cells is variable.

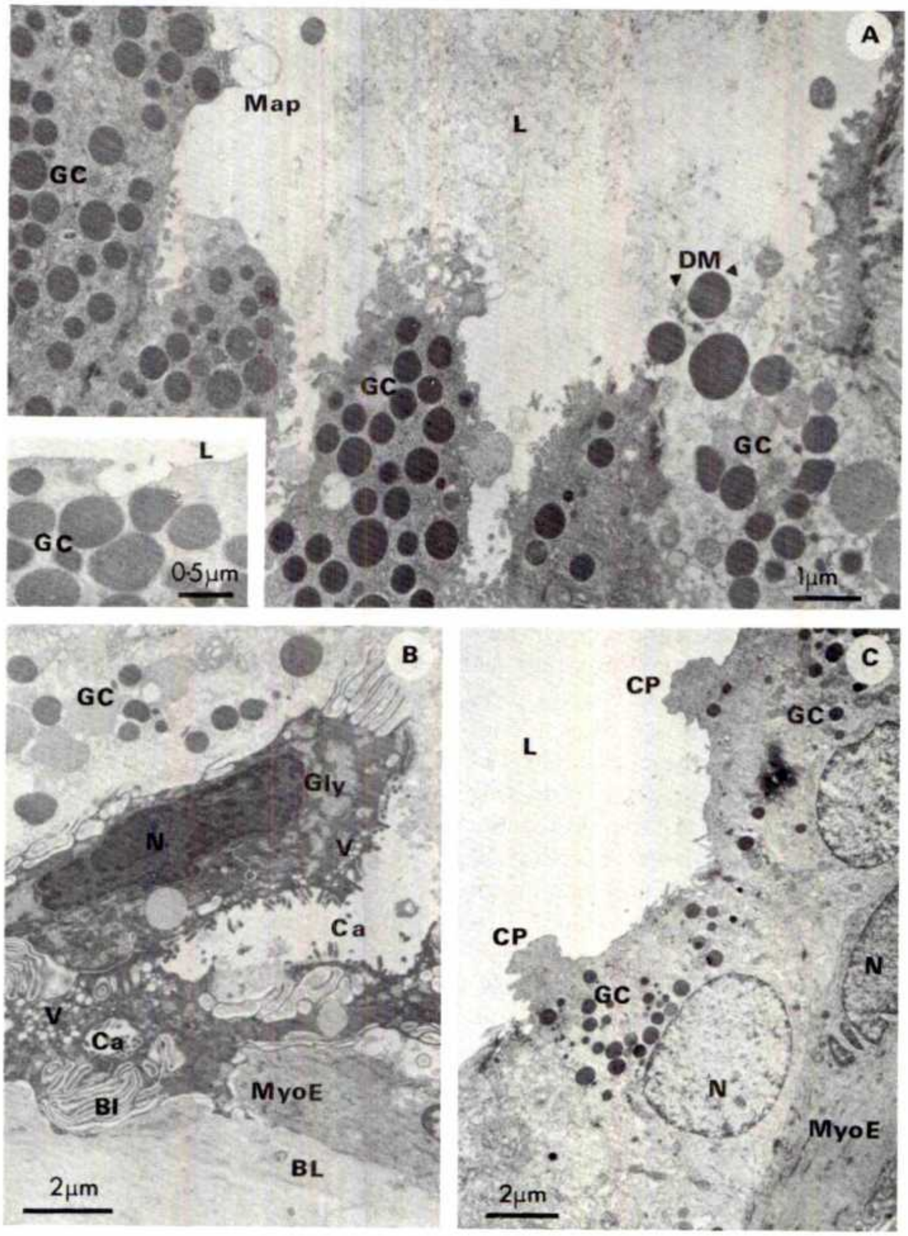

FIGURE 4. (A) The luminal surface of a fundus at the onset of sweating illustrating the probable modes of granule transport to the lumen. The insert shows a commonly observed appearance where the luminal membrane forms an open cup, most likely the result of exocytotic granule release. However, granules were in some instances, as shown, exuded from disrupted cells along with the cytoplasmic contents. Configurations reminiscent of a microapocrine process were also occasionally observed. (B) A dark cell containing lipid droplets, small vesicles and abundant glycogen, adjoining a canaliculus in the secretory epithelium of a fundus at the onset of sweating. Such cells were also present in samples taken after 3 h of continuous sweating and were occasionally detected in unstimulated glands. (C) Cytoplasmic projections at the luminal surface of granular cells during sweating. These were, however, observed in only a few instances.

appearance which still contained vesicles and lipid droplets were also occasionally found in the unstimulated gland.

The intercellular spaces between the now columnar granular cells, although also distended, were not as prominent. The short lengths of RER within the cytoplasm of these cells had dilated, but the Golgi apparatus was still not prominent. In all of these cells there was an accumulation of the now more spherical granules at the apical surface (Fig. 4a) and in some there was evidence of granule depletion (Fig. 4c). A limiting membrane was observed more easily around the paler granules than around the darker ones in neighbouring cells. Granule release to the lumen appeared to be mainly by a process of exocytosis (insert, Fig. 4a). Although no granules were caught at the moment of liberation, frequent examples of configurations consistent with this secretory process were seen. However, there was also evidence of granule loss by a microapocrine process (Fig. 4a) and some were disgorged into the lumen with other cytoplasmic constituents after complete disruption of the luminal membrane (Fig. 4a). Cytoplasmic protruberances at the luminal surface of these cells (Fig. 4c) were also occasionally observed.

Prolonged sweating. After continued sweating for over 3 h the gland appeared much less contracted; the lumen had considerably increased in size and the vacuolization had become more pronounced (insert, Fig. 5c). The myoepithelium, although still taller than in control glands, lacked basal crenations, but still contained glycogen. The secretory epithelium was apparently less stratified and the cells cuboidal. The now wider lumen contained cellular debris including lengths of membrane. The non-granular cells were by this time almost completely devoid of glycogen and their mitochondria were pale. The spaces and canaliculi between them were perceptibly narrower than those of glands at the onset of sweating, although still wider than in control glands (Fig. 5a). These cells, however, maintained their contact with each other and the basal lamina and still appeared to be actively secreting. The cells adjoining canaliculi, and rich in glycogen (Fig. 4b), were also found in sections taken at this stage of activity. The RER of the now cuboidal granular cells was much less dilated and the Golgi apparatus was not prominent. There were, however, still signs of granule transport to the lumen by exocytosis and occasionally by microapocrine secretion. The granules were again concentrated at the luminal region of the cell and although the number present in some cells was apparently reduced there was no evidence of complete loss of granules. Cells containing dark granules and others containing mainly paler ones were still observed.

Within the secretory epithelium, interspersed between the different cell types at irregular intervals, were dying and dead cells or their vestiges (Figs 5b, c). These resulted in gaps in portions of the epithelium, extending from the lumen to the basal lamina (Fig. 5c). It proved impossible to determine with certainty whether these resulted from the death of granular or non-granular cells or both.

DISCUSSION

The observed light and electron microscopical appearance of the fundus of the atrichial sweat gland before stimulation was in accord with previous findings (Dobson *et al.*, 1958; Ellis, 1962, 1965; Hashimoto, Gross & Lever, 1966a, b; Montagna & Parakkal, 1974; Hashimoto, 1978; Kurosumi *et al.*, 1982). Light microscopy also revealed heat-induced changes identical to those described by Dobson *et al.* (1958), even although the skin samples for the present study were taken immediately after local anaesthesia. Shortly after the onset of sweating the fundus was contracted and the lumen was smaller. Later the secretory epithelium became thinner and

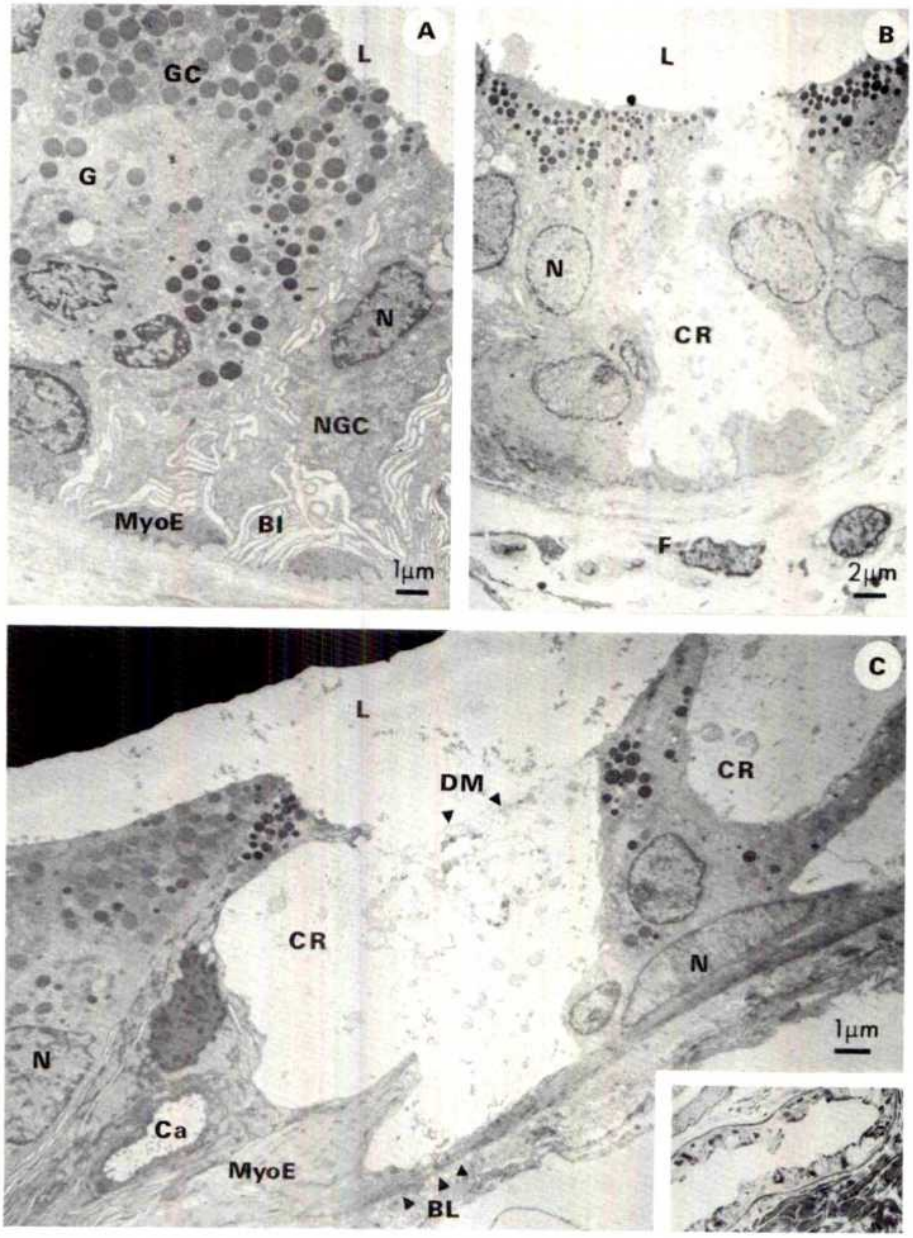

FIGURE 5. (A) The epithelium of the fundus after 3 h of sweating. Granules are still present in the granular cells and although the basal infoldings between non-granular cells are more dilated than in controls, they are not as distended as those observed at onset. (B) In this section from the fundus after 3 h of sweating, although there is some evidence of granule depletion, the basal infoldings are again less prominent than at onset. A noticeable feature of the secretory epithelium is the presence of an atretic cell, which in this instance, still has a luminal membrane. (C) Such atretic cells and their remnants were regularly observed within the secretory epithelium at this stage of activity even by light microscopy (insert). High-power examination revealed that these atretic cells disintegrate and lose their apical *zonulae occludentes* and luminal cell membranes. This degeneration causes gaps in the secretory epithelium, where the basal lamina is exposed to the lumen and hence a loss of epithelial integrity results.

exhibited prominent vacuolization. At this stage the lumen was wider and the gland appeared to have dilated. The human gland, therefore, differed from those of the cow, sheep, goat and horse, the fundi of which appear to remain contracted during sweating (Jenkinson *et al.*, 1979; Montgomery *et al.*, 1982b). Electron microscopy demonstrated that the gland was seemingly contracted in all specimens examined during the early period of sweating and later was always dilated. Thus, as in the human epitrichial gland (Bell, 1974) and those of the domestic animals, no evidence of synchronized periodic contraction of the myoepithelium was obtained. The principal role of what in the human atrichial gland seems to be a loosely woven basket of cells, with well-developed myofilaments and sparse interconnections (Ellis, 1965), may therefore be the provision of structural support for the secretory epithelium, as suggested by Montagna & Parakkal (1974) and Sato *et al.* (1979).

The results demonstrate that human sweat like that of domestic animals (Jenkinson *et al.*, 1979; Montgomery *et al.*, 1982b) is formed not only by secretion but also from the products of cell death. Two of the secretory processes exhibited by the human gland, fluid transport and vesicle exocytosis are common to the animal species studied to date and the third, microapocrine secretion, also occurs in the horse (Montgomery *et al.*, 1982b). The comparative data, therefore, illustrate that the fundamental processes of sweat formation in the human gland are the same as those in haired animals and it can be postulated that in general, sweat is formed from the products of:

(1) Secretion, mainly by fluid transport and vesicle exocytosis but in some species also by a microapocrine process;
(2) Cell death.

The contribution and efficiency of the different secretory processes clearly vary between species and in man cell specialization appears to have evolved. Evaluation of the contribution of degenerate cells to sweat composition awaits comparative information on secretory cell production and turnover in both the unstimulated and active gland. However, the morphological evidence, while again illustrating species variability, indicates that in some instances at least cell death is either induced or accelerated upon glandular stimulation. The resulting influence on sweat composition is likely to be significant particularly in man, in view of the consequent disruption of the secretory epithelium.

The occurrence of cell death and microapocrine secretion in the human sweat gland makes its classification as 'eccrine' untenable, and there is no longer any justification for the categorization of sweat glands as 'apocrine' or 'eccrine'. They also cannot be classed as 'holocrine' since degenerate cells contribute only a proportion of the sweat constituents. Even 'partly holocrine' is an unacceptable description, since it is not yet known if in all species the cell degeneration is a true secretory process induced or accelerated by stimulation or simply a manifestation of an associated mechanism of cell replacement. Moreover, in the species where increased cellular disruption follows stimulation, some of the cells, at least, almost certainly secrete vesicles before degeneration and hence contribute to the sweat more than their cell contents after disruption as in holocrine secretion. The classification of sweat glands therefore requires reappraisal and until more detailed information on the processes involved in sweat formation is forthcoming, it would seem prudent to abandon the present misleading categories 'apocrine' and 'eccrine' and revert to anatomical definitions. The terms epitrichial and atrichial (Bligh, 1967) adequately distinguish glands associated with hair follicles from those which open directly to the epidermis and, if applied, provide the basis for fresh assessment of sweat gland physiology.

The intercellular spaces between non-granular cells and the blind-ended canaliculi which may well, as in many other epithelia, generate a standing ionic gradient (Diamond & Bossert, 1967) were, at the onset of sweating, more distended in some regions of the epithelium than in others, suggesting variability in the response to stimulation. After continued sweating for over 3 h there was no decline in fluid output, but an observable narrowing of the intercellular spaces occurred between non-granular cells. Such morphological changes may presage the eventual secretory failure which Dobson *et al.* (1958) described in instances of profuse sweating continued for longer periods than in the present study. A small number of cells adjacent to canaliculi, which have mitochondria typical of the non-granular cells, contain aggregations of glycogen and appear to be less active. Their role, if any, in the epithelium is not known. They could represent non-granular cells in an early stage of degeneration, which have failed to respond to stimulation. However, the nuclei of those observed were not degenerate and their membranes were still intact. The presence of vesicles, lipid droplets and dilated RER in their cytoplasm is suggestive of activity and it is therefore conceivable, since they are also present in the unstimulated gland, that they are specialized secretory cells.

The granular cells showed less intercellular dilatation and are possibly more concerned with granule production and secretion than with fluid transport. These processes, which in domestic animals are undertaken by one secretory cell type (Jenkinson *et al.*, 1979; Montgomery *et al.*, 1982b), may well therefore have been largely separated in the human atrichial gland and delegated to specialized cells. The response of the granular cells to stimulation was not uniform; some exhibited a greater degree of degranulation than others. All, however, demonstrated granule accumulation at the luminal membrane of the cell. The most likely source of the secretory granules is the Golgi apparatus (Kurosumi *et al.*, 1982). Although the paler granules had a density comparable to their associated mitochondria, no evidence of mitochondrial transformation, as observed in cattle (Jenkinson *et al.*, 1979), was found. The evidence particularly from the active gland supports the conclusion (Inoue, 1979; Kurosumi *et al.*, 1982) that granule transport to the lumen of human sweat glands is by exocytosis; diacrine secretion (Hashimoto, 1978) was not observed. Granules also appeared on occasion to be released from the active cell by a microapocrine process, and some were disgorged, along with other cytoplasmic constituents and nuclei, from cells with disrupted luminal membranes. The cytoplasmic protrusions, occasionally seen at the luminal surface of active cells possibly indicate cytoplasmic extrusion by a microapocrine process but are more likely to be luminal membranes proceeding to eventual disruption and perhaps distorted into blisters by fixation (Shelton & Mowezko, 1978).

Cell remnants and even gaps were regularly found within the secretory epithelium after 3 h of continued sweating. It proved impossible by electron microscopy to identify accurately the cell types from which these remnants were derived, since the remaining organelles were disorganized and distorted. They are, however, unlikely to be solely the remains of disrupted granular cells. Dobson (1960), using light microscopy, observed atrophy of the 'large pale cells' after repeated episodes of profuse sweating and the present data do not preclude this possibility. The vacuolation of the secretory epithelium observed by light microscopy (Dobson *et al.*, 1958) is therefore most likely caused by disruption and loss of both granular and non-granular secretory cells.

Electron microscopy therefore clearly illustrates that during a single exposure to heat, portions of the secretory epithelium are breached, exposing the basal lamina. In these regions luminal tight junctions have been eliminated and there is no visible barrier to fluid exchange with the surrounding connective tissue. Since general distortion of the secretory coil and cellular atrophy is still apparent by light microscopy 24 h after heat-induced sweating (Dobson

& Lobitz, 1958) it would seem that epithelial cell replacement is slow and cell contacts are not rapidly re-established. In this respect the human atrichial gland appears to differ from those of domestic animals, in which neighbouring cells rapidly interconnect after cell death during sweating and prevent the passage of intercellular lanthanum into the lumen (Jenkinson, *et al.*, 1983).

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Structural changes in axillary eccrine glands following long-term treatment with aluminium chloride hexahydrate solution

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SUMMARY

Axillary skin biopsies from fifteen patients with axillary hyperhidrosis who had received long-term treatment with aqueous aluminium chloride solution were examined histologically. The apocrine glands were normal, but the eccrine glands showed conspicuous morphological changes of varying severity. These included vacuolization of the secretory epithelium, dilatation of eccrine acini with atrophy of secretory cells, and accumulation of PAS-positive, diastase-resistant material in the dilated lumen of the secretory coils. The histological changes correlated in most patients with decreased secretion of sweat. We conclude that long-term blockage of the distal acrosyringium due to aluminium salts may lead to functional and structural degeneration of the eccrine acini.

Aluminium chloride hexahydrate is widely used as the active ingredient in antiperspirant preparations. Its efficacy has been proven (Shelley & Hurley, 1975; Scholes *et al.*, 1978; Bandrup & Larsen, 1978; Hölzle, 1983) and its basic mechanism of action has been established (Reller & Luedders, 1977; Hölzle & Kligman, 1979a; Quatrale *et al.*, 1981). It has been found that aluminium and other metallic compounds with antiperspirant properties cause mechanical obstruction of the eccrine sweat duct within the acrosyringium. Hölzle & Kligman (1979a) suggested that metal ions form precipitating complexes with mucopolysaccharides and cause damage to luminal epithelial cells, thus generating an obstructive conglomerate completely plugging the acrosyringium. The secretory portion of the eccrine gland is not affected primarily by the metallic salt and remains active as is vividly demonstrated by the eruption of miliaria under heat stress in sites rendered anhidrotic by aluminium chloride solution.

Since patency of the epidermal sweat duct is restored by renewal of the acrosyringium due to epidermopoiesis, treatment of hyperhidrosis requires repeated exposures to aluminium

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chloride for prolonged periods of time. We have monitored a large group of axillary hyperhidrotics during long-term treatment with aluminium chloride solutions (Hölzle, 1983). The severity of hyperhidrosis seemed to decrease during the treatment period and this was reflected by a marked decrease in the frequency of treatments required to control the hyperhidrosis. This observation suggested that long-term application of aluminium salt solutions might alter the secretory portion of the eccrine glands functionally or structurally.

The present study was designed to investigate possible structural changes in axillary eccrine glands due to long-term aluminium chloride applications.

METHODS

Out of a group of 158 patients treated for axillary hyperhidrosis fifteen patients were arbitrarily selected for histological examination. These were seven females and eight males aged 24 to 60 years (mean 39·7). The only precondition for entering the study was that the patient had successfully treated the hyperhidrotic condition for at least 6 months before study. Hyperhidrosis was treated by overnight applications of 15% to 20% aluminium chloride hexahydrate in 2% methylcellulose as described previously (Hölzle, 1983). Individual treatment schedules varied from thrice weekly to applications once every 2 to 3 weeks (mean every 6·5 days). Treatment periods ranged from 6 to 40 months (mean 26·5 ± 12·5).

Small elliptical skin excisions about 1·5 cm in length were performed under local anaesthesia. All biopsies were taken from the lateral periphery of the axillary vault but still within the hair bearing area. Skin specimens were formalin-fixed, embedded in paraffin, serially sectioned and stained with haematoxylin–eosin and PAS.

Ten patients in whom excision of axillary skin for surgical treatment of hyperhidrosis was performed served as controls. Skin specimens were processed histologically as described above.

RESULTS

Controls. In all control patients who had not received aluminium chloride the axillary eccrine glands appeared normal. Very rarely, the lumina of acini and dermal ducts seemed to be slightly dilated.

Effects of aluminium chloride. The apocrine glands were found to be unchanged in all patients examined.

Within the acrosyringium of eccrine glands the characteristic plugs were found accompanied by necrosis of epidermal cells lining the ducts. Rupture of dermal or epidermal eccrine ducts was not observed. Apart from these acute effects of aluminium chloride on the distal portion of the glands, pronounced structural alterations of the eccrine acini were to be seen.

Subtle structural alterations which could be discerned were widening of the lumina of eccrine acini and dermal ducts and vacuolization of secretory cells (Fig. 1). The clear vacuoles were intra- as well as intercellular. As these changes became more prominent more eccrine glands were involved. The extent of structural alterations varied in one given skin specimen among different eccrine units. As damage increased, dilation of the secretory portion became more conspicuous and the acini appeared irregular and seemed to lose their structural integrity. Secretory cells appeared to undergo atrophy.

In severely altered eccrine glands the acini were grossly dilated and the secretory epithelium formed a thin layer which appeared stretched and atrophic. Some acini were filled with a

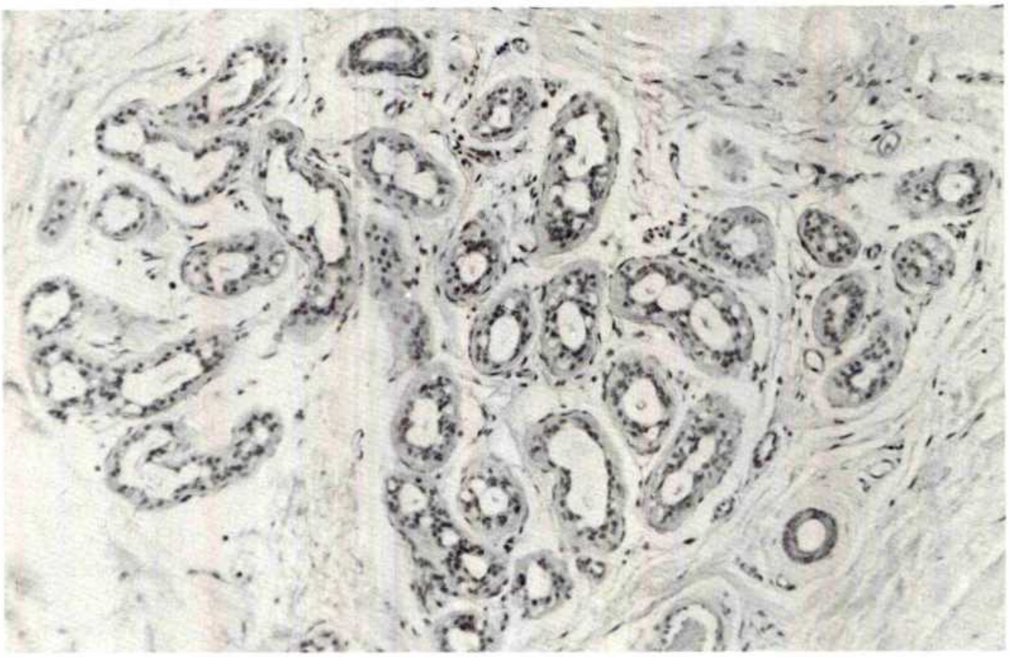

FIGURE 1. Eccrine acini showing vacuolization of the secretory epithelium and irregularly dilated lumina (H & E, × 115).

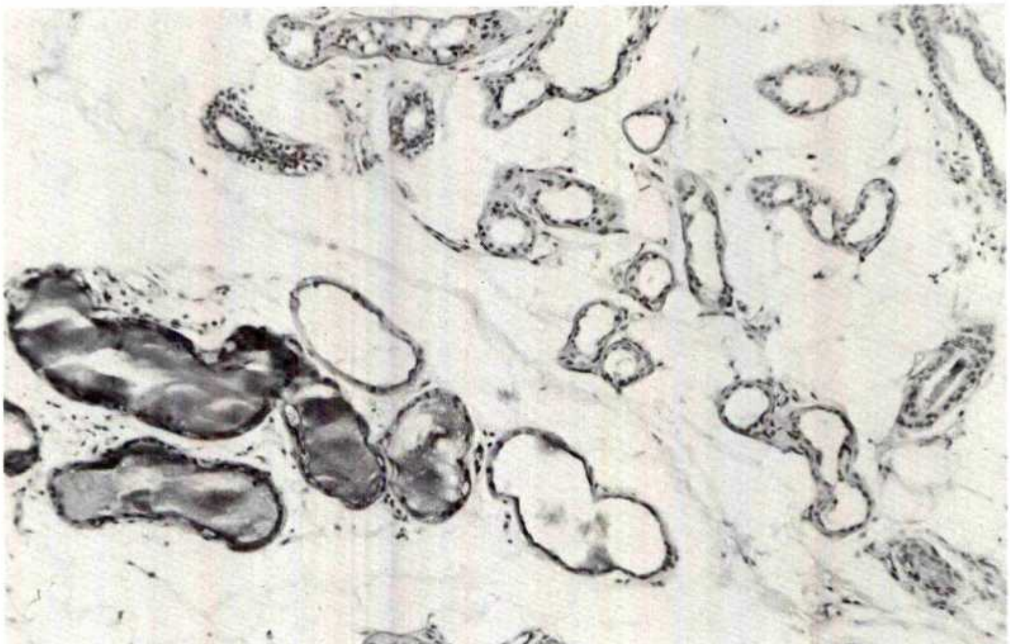

FIGURE 2. Grossly dilated and atrophic acini filled with PAS-positive material next to moderately affected eccrine gland (H & E, × 115).

PAS-positive, diastase-resistant, amorphous material (Fig. 2). In this stage, the eccrine secretory portions resembled apocrine acini depleted of their secretion. Again, glands were not equally affected. In most specimens, beside severely altered glands, moderately changed and sometimes normal eccrine units were detected.

A synopsis of the data is given in Table 1. Among the fifteen patients examined six showed severe disintegration of glandular structures, in four patients moderate changes were found and in the remaining five individuals only mild alterations of eccrine glands were seen. No correlation was found between the severity of the histological changes and the duration of treatment. Also the amelioration of hyperhidrosis as reflected by the increase of treatment intervals did not seem to be closely related to the structural changes seen histologically.

DISCUSSION

It is generally thought that the active secretory process of the eccrine glands creates an osmotic pressure of 300 to 500 mmHg. The myoepithelial cells forming a network around the secretory portion of the glands give the acini sufficient structural support to withstand this strain. Experimental anhidrosis due to aluminium chloride on back or forearm skin (Hölzle & Kligman, 1979a) as well as in the axilla, as shown in the present study, failed to reveal rupture of the eccrine ducts. Therefore, it must be concluded that sweat formed by the secretory portion either leaks into the dermis without rupture of the ducts or is completely dammed back. In both instances the intraluminal hydrostatic pressure will rise and it seems that the secretory portion cannot withstand the increased pressure over sustained periods of time. Apparently, chronic sweat retention finally leads to dilation and atrophy of the eccrine acini with loss of secretory function.

However, susceptibility to structural disintegration seems to differ among individuals as well

TABLE 1. Synopsis of experimental and histological findings during long-term treatment with topical aluminium chloride hexahydrate

Patient sex and age	Duration of treatment (months)	Treatment intervals at beginning/end of treatment (days)	Degree of structural alteration of eccrine acini
F, 36	40	3/10	+ +
F, 38	39	2–3/21	+
F, 45	39	2–3/10	+ + +
M, 24	38	4/7	+
F, 37	36	4–5/4–5	+ + +
M, 40	36	3–4/14	+ +
F, 46	36	3–4/14	+
F, 52	29	5/5	+ +
F, 41	24	2–3/4–5	+ + +
M, 26	23	2–3/14	+ + +
M, 31	18	2/3–4	+ + +
M, 46	18	2–3/5–6	+ + +
M, 35	10	7/7	+
M, 39	6	2–3/14–21	+ +
M, 60	6	2–3/7	+

as in various glands within a given patient. This heterogeneity in the resistance of eccrine acini probably accounts for the failure to correlate the degree of structural alterations with duration of treatment or with reduction of sweat rates as therapy is continued over longer periods.

It is not surprising that apocrine axillary glands were not altered by aluminium salts. Metallic salts and some other water-soluble irritants selectively damage the eccrine acrosyringium, thus generating an occlusive plug in the distal eccrine ducts (Hölze & Kligman, 1979b). The apocrine ducts remain patent. Even when complete anhidrosis is induced in the axilla by use of aluminium chloride solution one can observe secretion of apocrine sweat upon intradermal injection of adrenalin (unpublished observation).

Although hyperhidrotic patients are pleased to find their condition improving during long-term treatment, the consequences of the present findings are not yet completely understood. Further studies on the kinetics and reversibility of the observed changes are necessary.

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Psoriasis: a defect in the regulation of epidermal proteases, as shown by serial biopsies after cantharidin application

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SUMMARY

The possible role of epidermal serine proteases in the genesis of psoriatic lesions was investigated by sequential biopsies of the epidermal damage induced by topical cantharidin. In the skin of normal subjects, epidermal damage was followed by the transient appearance of proteolytic activity in the upper epidermis accompanied by temporary hyperacanthosis and perivascular inflammatory cells in the superficial dermis. In the uninvolved skin of five patients with psoriasis this proteolysis persisted longer, for more than 7 days. Thereafter, in three of the patients, the proteolysis abated, and this was followed by disappearance of the hyperacanthosis and the dermal infiltrate; in the other two psoriatics the proteolysis and hyperacanthosis increased, and a typical Koebner phenomenon ensued. Migration of neutrophils into the epidermis occurred as a later event. Thus the abnormal persistence of proteolytic activity in the upper epidermis after cantharidin application distinguishes the normal from the psoriatic skin injury response and might initiate the psoriatic lesion.

Many biochemical abnormalities have been found in psoriatic lesions (Krueger, 1981), but their relative roles in the pathogenesis of psoriasis remain obscure. To be considered as the possible intiator of the psoriatic lesion, a biochemical abnormality should fulfil the following conditions: (1) it should be able to modify epidermal cell proliferation and differentiation and also to induce inflammatory cell migration into the epidermis; (2) it should be detected in psoriatic lesions and in the uninvolved skin of psoriatics in the active phase of the disease; (3) the abnormality should be inducible in uninvolved psoriatic skin and be detected in the earliest phases of the Koebner phenomenon.

A defect in the regulation of serine proteinases in the epidermis of psoriatics seems to fulfil the first two conditions. Proteases are able to induce cell proliferation and to modify cell differentiation (Christman, Silverstein & Acs, 1977; Hubbard, 1978; Lacombe & Hanoune,

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1979) and serine proteases were found to be in increased concentration in psoriatic lesions (Fraki, Briggaman & Lazarus, 1983). Moreover some proteases are able to activate complement and then to induce neutrophil accumulation (Lazarus, Yost & Thomas, 1977). Recently, we have found an abnormal proteolytic activity, inhibited by serine protease inhibitors and expressed in the keratinocytes of the upper stratum spinosum, in nearly all psoriatic lesions but also in the uninvolved skin of one third of the psoriatics studied (Dubertret *et al.*, 1982).

In the present work we used cantharidin, an acantholytic agent (Stoughton & Bagatell, 1959), to produce epidermal damage. We then compared the 'injury response' of normal skin and uninvolved psoriatic skin by sequential biopsies. Special attention was given to the presence of proteolytic activity in the upper stratum spinosum and to the relationship between this activity and the appearance of a Koebner phenomenon.

METHODS

A filtered alcoholic solution of cantharidin (0·1 g/ml) (Chang & Yao, 1976) was freshly prepared and was applied for 1 h, under occlusion, to the uninvolved skin of patients with psoriasis usually on a forearm, and on skin of the same area in normal controls.

Punch biopsies measuring 4·0 mm in diameter were taken after local anaesthesia with 2% xylocaine without adrenaline, overlapping the cantharide blister area and the surrounding skin. The biopsies were performed 2 days after the cantharide blister test in seven controls and eight psoriatics, and also 7 days after the cantharidin blister test in five controls and five psoriatics. Further biopsies were performed in the five psoriatics, who were sufficiently motivated to co-operate for the entire experiment, until epidermal healing or diagnosis of Koebner phenomenon by clinical and histological criteria.

Each biopsy was freshly cut into slices of 0·7 mm thickness as previously described (Dubertret *et al.*, 1983). One slice was put directly into Karnovsky fixative (Karnovsky, 1967) for cytological and morphometric analysis. One slice was incubated for 1 h at 37°C in Hank's medium in order to look for epidermal proteolytic activity using the skin as its own substrate (Dubertret *et al.* 1982). A last slice was incubated for 1 h at 37°C in Hank's medium containing phenylmethane sulphonide fluoride (PMSF, Sigma) 1 mg/ml and N-α-tosyl-L-lysine chloromethyl ketone (TLCK, Sigma) 3·7 mg/ml, known inhibitors of the epidermal serine proteinases found in psoriatic lesions (Dubertret *et al.*, 1982).

After incubation in the presence or absence of inhibitors, skin slices were fixed in Karnovsky fixative, postfixed in osmium tetroxide, embedded, cut in 0·5 μm sections of large surface area and processed for light microscopy as previously described (Dubertret *et al.*, 1980). The index of papillomatosis (IP), i.e. the ratio of the length of the epidermal basement membrane to the length of the corresponding stratum corneum, was measured with a Nachet 1000 S image analyser, and taken as an objective measurement of the epidermal acanthosis (Christophers, Parzefall & Braun-Falco, 1973).

RESULTS

An increased proteolytic activity, as found in spontaneous psoriatic lesions (Dubertret *et al.*, 1982), was observed under the stratum corneum in the seven normal volunteers and in the eight psoriatics 2 days after the cantharidin blister test (Table 1). This proteolytic activity was not observed in the early stages of epidermal healing but only when the upper epidermal cell layers

TABLE 1. The occurrence and extent of proteolysis in the upper stratum spinosum after epidermal damage induced by cantharidin

	Days					
	2	7	9	11	12	14
Psoriasis patients						
P1	C + IP = 1					
P2	A + —					
P3	B + + + + IP = 1·2					
P4	B + IP = 1					
P5	C + + IP = 1·1					
P6	A + + + IP = 1·3					
P7	A + + —	C + + IP = 1·2	C + IP = 1·5		O IP = 1·6	O IP = 1·4
P8	A + + + —	C + + + IP = 1·8	O IP = 1·4			
P9		B + + + IP = 2·0	B + + + IP = 2·2	O IP = 1·9		
P10		B + IP = 1·4	B + + + + IP = 1·8	Koebner		
P11		B + + + + IP = 2·0	A + + + IP = 2·2	Koebner		
Normal controls						
T1	A + + —					
T2	B + + + IP = 1·3					
T3	B + + + IP = 1·4	O IP = 1·4				
T4	A + + + IP = 1·3	O IP = 1·5				
T5	A + + IP = 1·1	O IP = 1·4				
T6	A + + + IP = 1·1	B + + IP = 1·5				
T7	B + + IP = 1·2	O IP = 1·2				

A = continuous proteolysis under the stratum corneum.
B = discontinuous proteolysis.
C = proteolysis associated only with isolated cells.
O = absence of proteolysis.
IP = the index of papillomatosis.

The proteolysis was observed in four or more (+ + + +), three (+ + +), two (+ +) or one (+) keratinocyte layers, immediately under the stratum corneum.

reappeared. This proteolysis was inhibited by TLCK plus PMSF. When possible the index of papillomatosis was calculated (Table 1) and the medium normal value was 1·2.

Sequential biopsies showed that this proteolytic activity disappeared after 7 days in four out of five normal volunteers tested (Table 1). In one volunteer a slight proteolysis persisted after 7 days, but it should be noted that the blister site was accidently injured and remained red and crusted in this subject at the time of biopsy. Among these five volunteers (T3–T7) a slight parakeratosis was observed in T4 and T6 as well as a slight increase of the index of papillomatosis in T4–T6 (Table 1). By contrast, in the five psoriatics studied, this proteolysis was still observed after 7 days, and the index of papillomatosis was slightly higher at this time in psoriatics (medium value 1·7) than in controls (medium value 1·4).

In subsequent biopsies the progressive disappearance of the proteolysis and a parallel decrease of IP was observed in three psoriatics (P7, P8 and P9). In the two other patients (P10 and P11) an increase of epidermal proteolysis occurred with an increase of IP leading to a typical clinical and histological Koebner phenomenon (Table 1).

Slight perivascular inflammatory cell infiltrates were observed in the superficial dermis 2 days after the test in controls and in psoriatics. These perivascular infiltrates disappeared 7 days after the test in controls but were still observed in psoriatics.

Neutrophils and macrophages were observed in the cantharidin blister fluid 2 days after the test in psoriatics as well as in controls. However this migration of inflammatory cells into the epidermis was a transient phenomenon and, in 0·5 μm sections of large surface area, allowing precise cytological analysis, no inflammatory cells could be observed in the epidermis in biopsies taken later than 2 days. Munro micro-abscesses were observed as a very late phenomenon in patient P11, and in one other Koebner-positive psoriatic who was studied only for inflammatory cell infiltration and not for epidermal proteolysis.

DISCUSSION

The induction of proteolytic activity, inhibited by serine protease inhibitors, that we have observed in the upper stratum spinosum during normal epidermal healing, might play a role in the regulation and differentiation of normal human epidermis. Epidermal injury is followed by epidermal cell proliferation and the production of chemotactic substances (Marks *et al.*, 1979), and proteases are able to induce cell proliferation (Christman *et al.*, 1977) and to activate chemotactic substances (Hatcher *et al.*, 1977). Thus the protease–antiprotease equilibrium in the upper stratum spinosum could well contribute to the epidermal homeostasis and be a part of the chalone regulation described in the epidermis (Bullough & Laurence, 1960).

Our study shows that this proteolytic activity, transient during normal epidermal healing, persists longer in psoriatics. This persistence is associated with a progressive increase of hyperacanthosis and the production of chemotactic stimuli as shown by the presence of inflammatory cells in the superficial dermis: the situation should be described as a chronic (or not inhibited) epidermal healing process. Moreover when the proteolytic activity was finally inhibited, the hyperacanthosis abated and the inflammatory cells disappeared from the superficial dermis; when a Koebner phenomenon occurred the proteolytic activity was not at all inhibited and was found in the Koebner psoriatic lesions as in all the other psoriatic lesions previously studied (Dubertret *et al.*, 1982). Thus the abnormal persistence of a proteolytic activity in the upper epidermal layers after epidermal wounding serves to differentiate the normal and psoriatic skin injury response and might initiate the psoriatic lesion.

Our study of the Koebner phenomenon obtained after epidermal injury shows that the neutrophil migration into the epidermis seems to be a late phenomenon. It is known that this neutrophil migration can be a consequence of the activation of complement (Tagami & Ofugi, 1977) by serine proteases in the upper layer of the epidermis (Hatcher *et al.*, 1977; Dubertret *et al.*, 1982); these activated neutrophils are able to release their lysosomal enzymes under the stratum corneum (Dubertret & Touraine, 1976) leading to chronic intraepidermal damage and possibly to the self-perpetuation of the disease.

In conclusion, a defect in the regulation of serine proteases in response to non-specific skin injury does exist in psoriatic epidermis and this defect could be a good candidate for the role of biochemical trigger for the psoriasis lesion. However the regulation of the protease–antiprotease equilibrium in human epidermis, the nature of the various proteases and antiproteases involved and the relationship of this defect to other abnormalities observed in psoriasis such as the defect of neutrophil function (Greaves, 1983), various immunological modifications (Beutner, 1982) and other biochemical alterations e.g. those of the leukotriene pathway (Brain *et al.*, 1983; Czarnetzki, Grabbe & Mardin, 1983) must be investigated before a firm conclusion can be reached.

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Metabolic changes in the psoriatic lesion during therapy

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SUMMARY

Three marker enzymes were measured during treatment of psoriatic plaques with two different therapies. During treatment with clobetasol propionate the epidermal enzymes (acid phosphatase and glucose-6-phosphate dehydrogenase) returned to normal within 14 days whereas capillary alkaline phosphatase remained at the original level. By contrast, all three marker enzymes reverted to normal at the same tempo during PUVA therapy, reaching the control range after 4–8 weeks.

Our previous investigations into the pathogenesis of the psoriatic lesion (van de Kerkhof *et al.*, 1983b,c) have established that abnormalities in the dermal capillary certainly precede, and may well be a mandatory precursor of, epidermal dysplasia. In this communication we present data regarding the levels of acid phosphatase (ACP; keratinizing zone), glucose-6-phosphate dehydrogenase (G-6-PDH; proliferation zone) and alkaline phosphatase (ALP; dermal capillary) during the therapeutically induced regression of the established lesion. We followed the dynamics of two different treatments: the application of a local corticosteroid and PUVA therapy.

METHODS

Experimental design for corticosteroid evaluation

Three patients with chronic stable plaque psoriasis participated in this study. None had ever received any systemic therapy for psoriasis, and none had used local therapy for at least 1 month prior to investigation. In each patient a lesion of 6 to 10 cm diameter was selected (two from the upper arm and one from the upper leg) and six prospective biopsy sites were 'mapped' on to paper prior to the experiment. All sites were at least 1 cm inside the margin of the lesion and 2 cm apart. Three healthy subjects (without history or signs of psoriasis) were employed as controls, an area of about 10 cm^2 being marked on the upper arm ('corticosteroid controls').

The selected lesions and the control areas were treated with clobetasol propionate 0·05% in an ointment base (Dermovate®, Glaxo) twice per day, applied thinly and evenly, without occlusion. Biopsies were taken free-handed, using a razor blade in conjunction with a metal guard (hole 4 mm diameter), immediately prior to the application of steroid and at subsequent intervals of 1, 3, 7, 11 and 18 days. Specimens were weighed and stored at −70°C prior to analysis.

Experimental design for PUVA evaluation

Four patients with widespread chronic stable plaque psoriasis were selected for this study. They received no treatment at all during 1 month prior to the initiation of PUVA therapy. In each patient twelve prospective biopsy sites were mapped on to paper from lesions localized on the upper arm. For comparison we used biopsies from the clinically uninvolved skin during the course of the therapy ('PUVA controls').

PUVA treatments were carried out in a Waldmann 6001 cabin (H. Waldmann and Co., Werk für Lichttechnik, Schwenningen) 2 hours following the ingestion of 8-methoxypsoralen (MOP) (Meladinine®, Basoderm). Dose schedules for 8-MOP and UV-A were as described by Wolff *et al.* (1977). Dose increments were 0·5–1·0 J/cm² set by the tolerance of the individual patient. We employed two treatments per week until the patients were essentially free from lesions. From then on a maintenance regime of one exposure per week was adopted. During this study patients were permitted to apply only bland emollients.

The biopsy technique was as described above. All specimens were taken immediately before UV-A exposure. During the first 2 weeks lesions were biopsied twice and thereafter once per week.

Untreated controls

During the course of these studies additional control biopsies were taken from the untreated skin of twenty subjects who had no signs or history of psoriasis.

Biochemical assays

All procedures were as described previously (van de Kerkhof *et al.*, 1983b). In brief, the specimens were homogenized in 1 ml aqueous bovine serum albumin solution and centrifuged. Duplicate 20 μl aliquots of the supernatant were used for fluorimetric determinations of the levels of ACP, G-6-PDH and ALP. DNA concentrations were measured in duplicate 50 μl aliquots for use as reference variable for the epidermal enzymes (ACP and G-6-PDH).

RESULTS

Clinical response to therapies

All three steroid-treated lesions showed an excellent clinical response. A marked but transient reduction in the erythema was observed (days 1–3), followed by a return to its original level; this persisted until the end of the experimental period (day 18). Scaling and thickening diminished rapidly from day 1 onward, and had essentially subsided by day 7 or 11. No clinical reaction of any kind was seen on the corticosteroid control subjects.

The four patients treated with PUVA were at the end of the experimental period (9 weeks) essentially free from lesions, the individual clearance times ranging between 4–8 weeks. A slight erythema remained at the sites of the previous lesions. The total UV-A dosage ranged from 35 to 144 J/cm². The clinically uninvolved skin showed no burning reactions necessitating UV-A dosage reduction. After 9 weeks of treatment all patients showed a slight pigmentation.

Metabolic response to therapies

The levels of our three marker enzymes are presented in Figs 1 and 2. All levels are expressed as percentage of the means of the untreated controls. The shaded areas represent the normal range (mean ± 1 s.d.) for each enzyme. This range is composed of our previous control group (van de Kerkhof *et al.*, 1983b) together with the twenty additional controls assayed during this investigation.

The activities of the three marker enzymes during corticosteroid evaluation are shown in Fig. 1. It is seen that the epidermal values (ACP and G-6-PDH) of the lesion decrease at a similar tempo, both reverting to the normal range after about 2 weeks of treatment. By contrast, the capillary marker (ALP) was uninfluenced by the steroid, remaining at the level of the original lesion during the whole investigation. The activities of all three enzymes in the steroid–control group remained within the normal range throughout the experiment.

The activities of the three marker enzymes during PUVA therapy are shown in Fig. 2. It is clear that all three enzymes reverted to normal with the same speed. The metabolic response closely paralleled the clinical response in each of the four patients. In the uninvolved skin (PUVA controls) the epidermal values remained within the normal range whereas the capillary marker ALP showed a small but consistent increase.

DISCUSSION

Our corticosteroid study is in good agreement with the literature regarding the clinical and histopathological response to this therapy. Marriot and Munro (1976), for example, reported clearing times (using the same corticosteroid preparation as we did) between 10–21 days. The histopathological literature agrees that the capillary and infiltrate involvement persist rather long after clinical and epidermal clearance (Suurmond, 1965; Braun-Falco, Thian Prasit & Kint, 1963). Our data permit a more definitive conclusion, namely that with epidermal

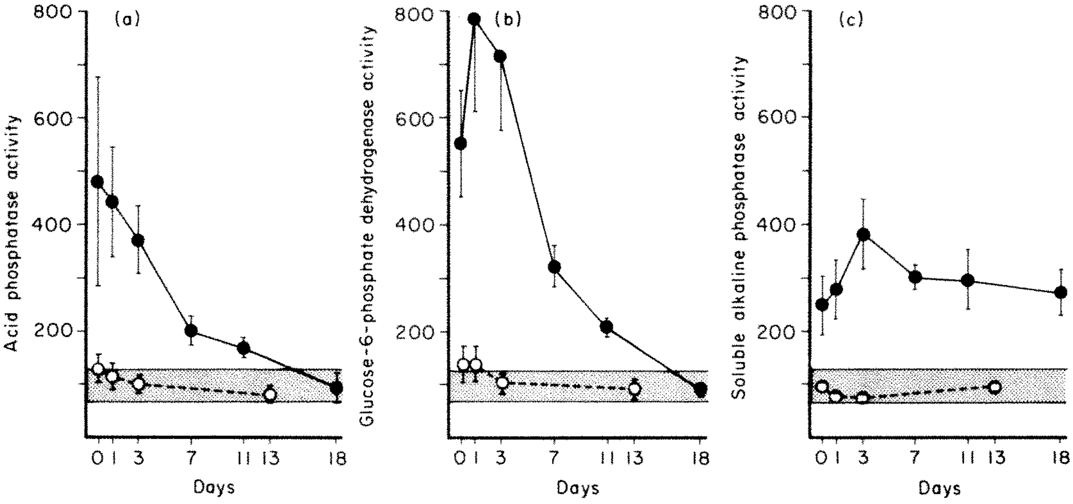

FIGURE 1. Enzyme levels during treatment with clobetasol propionate. All levels are expressed as percentage of the means of the controls. (a) ACP (b) G-6-PDH (c) ALP. Open circles represent the corticosteroid controls and filled circles the treated psoriatic lesions. Vertical bars are s.e.m. Shaded areas indicate normal ranges (mean ± s.d.).

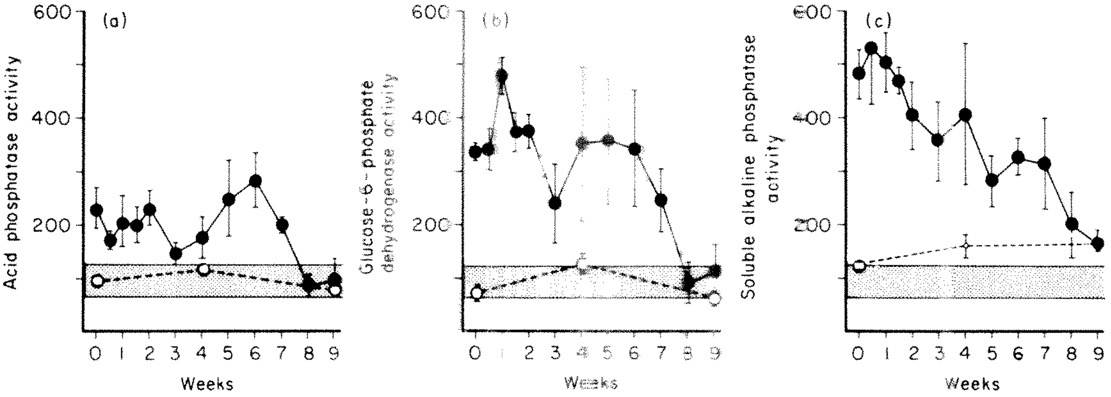

FIGURE 2. Enzyme levels during PUVA therapy. (a) ACP (b) G-6-PDH (c) ALP. Open circles represent the uninvolved skin (PUVA controls) and filled circles the lesions.

normalization (18 days) the capillary marker ALP remains in the range of the untreated lesion. These observations also support the concept that the epidermis is the direct 'target' for the anti-psoriatic effects of corticosteroids. However, the lack of response in healthy skin (Fig. 1, corticosteroid controls), makes it clear that the therapeutic effects are not mediated by a direct inhibition of G-6-PDH or ACP, despite reports that steroids are effective inhibitors of G-6-PDH *in vitro* (Raab & Siber, 1974; Cotton & van Rossum, 1974).

The clinical response to PUVA therapy was also in agreement with previous reports; for example Melski *et al.* (1977) reported clearing times (also using a treatment schedule of two exposures per week) of 9·7 weeks average. There is total disagreement in the literature about the order in which the different skin compartments normalize during PUVA clearance. From histopathological studies Braun-Falco, Hofmann & Plewig (1976) conclude that the keratinizing zone is the target for this therapy. Pulmann *et al.* (1976), employing autoradiographic techniques, reported that the primary action is upon epidermal proliferation. Braverman and Sibley (1982), using electromicroscopy, and Ryatt *et al.* (1982), using reflectance spectrophotometry, nominate the capillary as the target structure. Our enzyme studies clearly indicate that keratinizing activity (ACP), epidermal proliferation (G-6-PDH) and capillary metabolism (ALP) revert to normal at the same tempo. The slight elevation of the capillary marker in the clinically uninvolved skin during PUVA therapy is intriguing; a similar increase has been shown following whole spectrum UVR (van de Kerkhof *et al.*, 1983a).

Comparing the present data with our previous studies, we may note that three situations have now been encountered in which the capillary marker (ALP) is grossly elevated but the epidermal measurements are normal. These are: first, the 'normal' skin 0·4–2·0 cm outside the margin of the growing psoriatic plaque (van de Kerkhof *et al.*, 1983b); second, the uninvolved skin of the psoriatic patient following limited injury (van de Kerkhof *et al.*, 1983c); and finally, the established psoriatic lesion after about 2 weeks' corticosteroid therapy. Since it is well established that if treatment with corticosteroid is stopped abruptly at this point a 'rebound' usually occurs, these three models seem to correspond precisely to these situations where overt clinical change may be anticipated within a few days. Thus the evidence that capillary alterations are a mandatory precursor of epidermal dysplasia now seems overwhelming.

Although the link between the capillary changes and the epidermal response is of course speculative, the time-lag seems to rule out direct chemical mediation. A more likely possibility is that this period is required to establish the dermal infiltrate, which is in turn responsible for the epidermal hyperplasia. This view would suggest the infiltrate cells as a likely 'target' for PUVA, an idea compatible with the clinical observations that PUVA is of value in diseases such as pre-mycotic eruptions and mycosis fungoides, and also with recent laboratory evidence that cells of mesenchymal origin are exquisitely sensitive to this modality (Verhagen *et al.*, 1983).

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Immunological aspects of psoriasis. VI. Impairment of isoprenaline and theophylline-induced inhibition of mitogen responsiveness

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SUMMARY

Pharmacological abnormalities occur in the psoriatic epidermis, and if similar abnormalities occur in the peripheral blood mononuclear cells they could impair the immune responses in psoriasis. In a paired control study, we have tested the capacity of histamine, isoprenaline and theophylline (10^{-5} and 10^{-7} M) to inhibit the mitogen responsiveness of blood mononuclear cells from normal and psoriatic subjects, using phytohaemagglutinin and concanavalin A. In the normal controls, mitogen responsiveness was inhibited by all three pharmacological agents by about 30 to 50%. In cells from psoriatic patients, the response in the presence of histamine was inhibited (as in the controls), but isoprenaline caused no inhibition, and theophylline paradoxically increased the mitogenic responses. These results suggest there is a defect in the pharmacological response of the blood mononuclear cells in psoriasis.

Psoriasis is associated with a variety of immunological and pharmacological disturbances (Guilhou, Meynadier & Clot, 1978). Immunological abnormalities occur in the serum, epidermis and peripheral blood mononuclear cells (PBMC). T-cell numbers are decreased and there is an increase in null cells (Clot *et al.*, 1978). Mitogen responsiveness is significantly depressed in the presence of concanavalin A (Con A) and, to a lesser extent, phytohaemagglutinin (PHA). An imbalance of cyclic nucleotides and abnormal responses to β-adrenergic agents have also been described in the psoriatic epidermis (Voorhees *et al.*, 1972; Royer *et al.*, 1982; Adachi *et al.*, 1975; Marcelo *et al.*, 1979). A similar imbalance, if present in PBMC, could be important in modifying the behaviour of cells involved in the immune response, since this is modulated by cyclic nucleotide levels (Strom, Lundin & Carpenter, 1977).

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We have now studied the response of psoriatic PBMC to pharmacological agents acting on cyclic nucleotide metabolism, namely isoprenaline, histamine and theophylline. These agents inhibit the proliferative response to mitogens by increasing cellular cyclic AMP. Consequently, these drugs were tested on their ability to inhibit the proliferative response to PHA and Con A.

METHODS

Reagents

Theophylline and histamine were purchased from Sigma Chemical Company. Isoprenaline (isoproterenol) was obtained from Winthrop Laboratories. Solutions were made up in tissue culture medium RPMI-1640 (Flow Laboratories) which contains Ca^{2+} and Mg^{2+} ions.

Patients

Fourteen psoriatic patients were studied. There were twelve males and two females ranging in age from 21 to 79 years. All suffered from psoriasis vulgaris. Four patients had generalized psoriasis, seven had numerous disseminated lesions, and three had few scattered lesions. Twelve out of fourteen patients were in a progressing stage of the disease. Patients had received neither systemic therapy nor topical corticosteroids for 3 months prior to the study. The control group included 14 sex- and age-matched healthy volunteers. The blood total lymphocyte counts were in the normal range in both groups. The proportion of T lymphocytes, as assessed by E-rosettes, was lower than 60% in six out of fourteen psoriatic patients (in the control group, the mean percentage of E-rosettes was 73·5% ± 6·5 s.e.m.).

Peripheral blood mononuclear cells (PBMC)

PBMC from healthy adult volunteers and psoriatic patients were obtained by centrifugation on Ficoll-Hypaque density gradient. They were then washed three times in Hank's balanced salt solution and were resuspended at a concentration of 10^6 cells/ml in culture medium RPMI-1640, supplemented with 15% of fetal calf serum (Seromed).

After preliminary dose–response curve experiments using concentrations of pharmacological agents ranging from 10^{-4} to 10^{-9} M, two optimal concentrations reaching a plateau of response were chosen. PBMC were preincubated with these drugs as follows: 10 μl of solution were distributed (×20) in each well of microtitre plates (Falcon 3040). Then 190 μl of the cell suspension were added and the plates were incubated at 37°C for 30 min (histamine and isoprenaline) or for 60 min (theophylline). Control cultures were incubated for the same period with culture medium alone. Then, PHA-P (Difco) and Con A (IBF) were added at final concentrations of 50 μg/ml. Assays without lectins served as controls to determine the background stimulation.

Cultures with or without drugs were performed in quadruplicate, incubated at 37°C in an humid atmosphere of 5% CO for 3 days, and labelled for 16 hours with 1 μCi of [^{3}H]thymidine per well (specific activity 1 Ci/mM).

After cell harvesting, samples were counted for 4 min in a liquid scintillation counter (Intertechnique). The results were expressed as disintegrations per minute (d.p.m.) and as stimulation index corresponding to the ratio of d.p.m. of cultures in the presence of mitogens to the d.p.m. of unstimulated cultures. The effect of each drug was expressed as a percentage of variation calculated as follows: Percentage of variation = 1 − (stimulation index of cultures added with a pharmacological agent/stimulation index of control cultures) × 100.

Differences between healthy individuals and patients were evaluated by Student's t-test on paired values from each experiment.

TABLE 1. Results of fourteen experiments using peripheral blood mononuclear cells from the control group, showing the percentage of inhibition of lymphoproliferative responses to PHA and Con A by pharmacological agents

Pharmacological agents added (mol/l)	Per cent inhibition of the response to	
	PHA	Con A
Isoprenaline		
10^{-5}	−47·8 ± 12·2	−42·3 ± 22·4
10^{-7}	−52·1 ± 11·9	−39·2 ± 14·1
Histamine		
10^{-5}	−42·5 ± 10·8	−29·0 ± 11·9
10^{-7}	−38·7 ± 7·6	−49·6 ± 28·8
Theophylline		
10^{-5}	−25·4 ± 10·2	−34·7 ± 12·5
10^{-7}	−11·9 ± 6·4	−38·4 ± 15·2

The results were calculated as indicated in the text (Methods) and expressed as mean percentage of inhibition ± s.e.m. The minus signs indicate a negative percentage of variation, i.e. an inhibition.

TABLE 2. Results of fourteen experiments using peripheral blood mononuclear cells from the psoriatic group, showing the percentage of variation (inhibition or stimulation) of lymphoproliferative responses to PHA and Con A by pharmacological agents

Pharmacological agents added (mol/l)	Per cent inhibition of the response to	
	PHA	Con A
Isoprenaline		
10^{-5}	+2·9 ± 13·2	−14·5 ± 9·8
10^{-7}	+3·7 ± 14·9	+1·1 ± 10·7
Histamine		
10^{-5}	−38·7 ± 12·6	−29·0 ± 9·7
10^{-7}	−31·1 ± 14·8	−41·5 ± 8·1
Theophylline		
10^{-5}	+34·7 ± 24·0	+18·7 ± 20·2
10^{-7}	+54·0 ± 24·5	+39·7 ± 20·6

The results were calculated as indicated in the text (Methods) and expressed as mean percentage of inhibition ± s.e.m. The minus signs indicate a negative percentage of variation, i.e. an inhibition. The plus signs indicate a positive percentage of variation, i.e. a stimulation

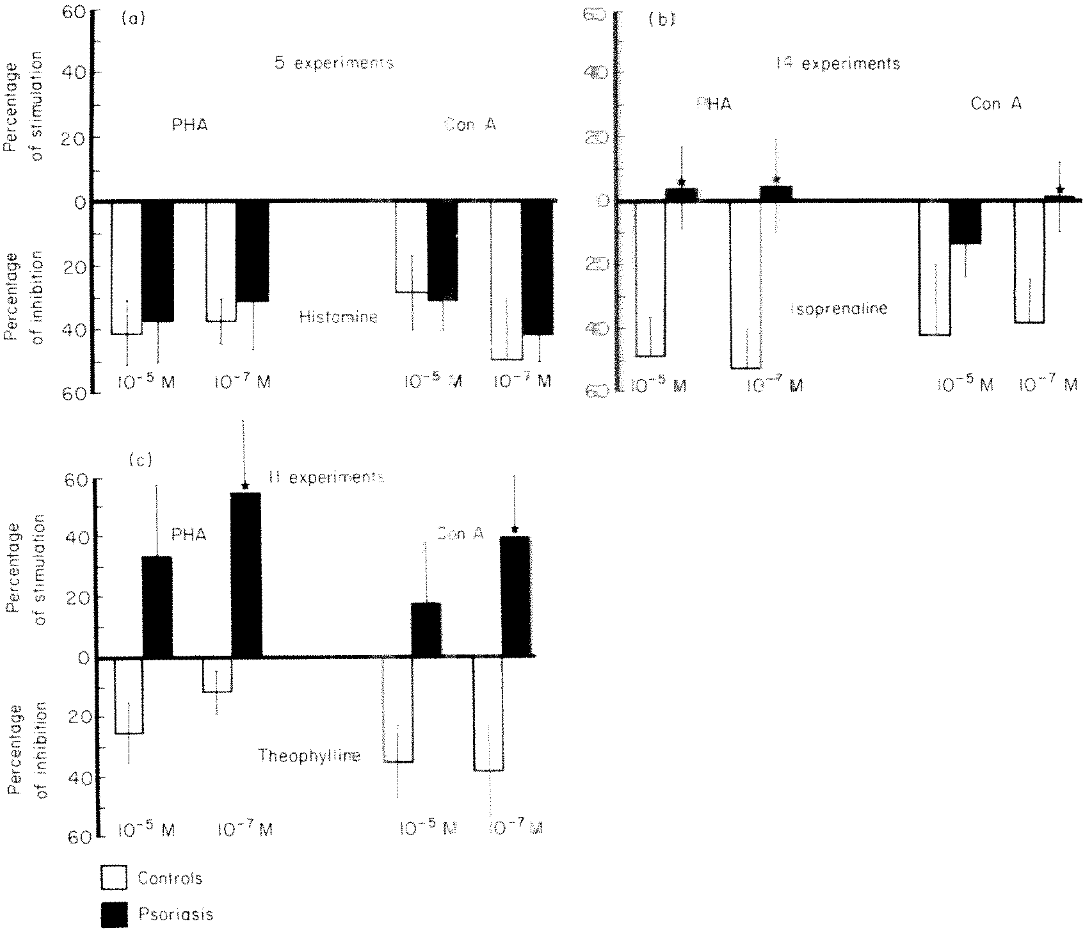

FIGURE 1. Effects of histamine, isoprenaline and theophylline on the lymphoproliferative response to T-cell mitogens of peripheral blood mononuclear cells from control subjects and psoriatic patients. Results are expressed as percentage of variation (stimulation or inhibition) as indicated in the text (Methods). The standard error of the mean is indicated by error bars. The comparison was made with a baseline corresponding to the proliferative responses to mitogens (PHA 50 μg/ml, Con A 50 μg/ml) observed when control or psoriatic lymphocytes were cultured without the drug. (a) Peripheral blood mononuclear cells were preincubated with 10^{-5} M or 10^{-7} M of histamine prior to the addition of mitogens. (b) Peripheral blood mononuclear cells were preincubated with 10^{-5} M or 10^{-7} M of isoprenaline prior to the addition of mitogens. (c) Peripheral blood mononuclear cells were preincubated with 10^{-5} M or 10^{-7} M of theophylline prior to the addition of mitogens. (*) Indicates a statistically significant difference ($P < 0{\cdot}05$) between the data from control subjects and those from psoriatic patients.

RESULTS

The results from the control group are given in detail in Table 1. An inhibition of mitogen responsiveness was observed when PBMC were preincubated with isoprenaline, histamine and theophylline.

The results from psoriatic patients are indicated in Table 2. The observed inhibition of

PBMC proliferative response due to histamine was the same as in controls (Fig. 1a). In contrast, isoprenaline inhibition was absent (Fig. 1b) and theophylline induced a paradoxical increase of mitogenic response to PHA and Con A (Fig. 1c). A more detailed analysis of the results showed a statistically significant lack of inhibition by adrenaline in 50% of patients and a statistically significant abnormal stimulation by theophylline in 70% of them. No relationship between these abnormal pharmacological responses and the clinical or immunological status of the patients could be demonstrated.

DISCUSSION

Mitogenic plant lectins induce lymphocyte proliferation by several different but obscure mechanisms which involve calcium influx and modifications of cyclic nucleotide equilibrium (Hadden & Coffey, 1982). These changes include an increase of cyclic GMP through activation of guanylate cyclase (Hadden & Coffey, 1982), and a decrease of cyclic AMP through inhibition of nucleotidase, with a consequent decrease in adenosine stimulation (Dornand, Bonnafous & Mani, 1980).

The inhibitory effect of cyclic AMP-increasing agents is well established in normal subjects and was confirmed in our study. The lack of such an inhibitory effect in psoriatic subjects indicates a defect of pharmacological reactivity of their PBMC. The level of the defect has to be determined, but several hypotheses can be raised in the light of our results.

Firstly, a defect of β-adrenergic receptors (as demonstrated in epidermal cells) could explain the lack of responsiveness to isoprenaline. This hypothesis needs confirmation by studies of adenylate cyclase responsiveness to β-agonists in terms of cyclic AMP production. However, the reported data on cyclic nucleotide levels in the β-stimulated PBMC of psoriatics, even if conflicting, argue for a normal response (Geerdink *et al.*, 1980; Harpaz *et al.*, 1980; Herlin & Kragballe, 1981; Hanson *et al.*, 1983). We have not found any significant difference between psoriatics and controls in a study of isoprenaline-stimulated adenylate cyclase activity (unpublished data).

On the other hand, the paradoxical effect of theophylline could be related to an impairment of different pathways of cyclic nucleotide metabolism. For instance, theophylline inhibits phosphodiesterase (PDE) and competes with adenosine for the same membrane receptors (Bonnafous *et al.*, 1982; Woolff, Londos & Cooper, 1981). Consequently, disturbances of cyclic nucleotide PDE or of adenosine receptor functions could explain the unexpected lymphocyte responsiveness to this pharmacological agent. Unfortunately, nothing is known about PDE and adenosine responsiveness of psoriatic lymphocytes. Moreover, a direct (cyclic nucleotide-unrelated) effect of theophylline on nucleic acid metabolism has been demonstrated (Steinberg & Whittaker, 1978) and this could be impaired in psoriatic lymphocytes.

Finally, different subsets of T lymphocytes can be identified, according to their sensitivity to theophylline. They could correspond to different stages of maturation, and/or to different subpopulations expressing either suppressor or helper functions (Limatibul *et al.*, 1978). The paradoxical potentiation of the mitogenic effect by theophylline in psoriasis could be related to abnormal theophylline-sensitive cell populations in this disease.

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Identification of the plasminogen activator secreted by human fibroblasts

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SUMMARY

Plasminogen activator was partially purified from cultured human fibroblasts. The enzyme binds to fibrin, has a molecular weight of 68,000 and is antigenically identical to human uterine tissue plasminogen activator.

Extracellular plasmin-dependent proteolysis is involved in a wide range of normal tissue remodelling processes such as mammary gland involution, menstruation, inflammation, embryogenesis and neovascularization (Ossowski, Biegel & Reich, 1979; Bonnar & Sheppard, 1983; Ryan, Nishioka & Dawber, 1971; Strickland, Reich & Sherman, 1976; Rifkin *et al.*, 1981). It has been suggested that plasminogen activator plays a role in normal squame detachment (Myhre-Jensen & Astrup, 1971) since keratinocytes show increased intracellular levels of the enzyme as differentiation advances (Isseroff, Fusenig & Rifkin, 1982) and cultured keratinocytes secrete small amounts of the enzyme (Atkinson *et al.*, 1982). Both plasminogen and plasminogen activator are present in the basal layer of epidermis (Isseroff & Rifkin, 1983; Green, 1977) where the enzymes are believed to play an important role in the development of acantholysis in pemphigus and psoriasis (Hashimoto *et al.*, 1983; Fraki *et al.*, 1983).

The formation of new capillaries is marked by the destruction of basal lamina and the invasion of cells through interstitial tissue, a process that also involves plasminogen activator and collagenase action (Gross, Moscatelli & Rifkin, 1983). During re-epithelialization of wounded skin, migrating fibroblasts and epidermal cells interact with a dense amorphous matrix composed in part of fibrin and cross-linked fibronectin (Clark *et al.*, 1982). Plasminogen activator secretion is a property exhibited by a variety of invasive cells, including malignant cells (Cederholm-Williams, 1981).

Though plasminogen activators are present in variable amounts in most human tissues the enzymes have only been isolated in substantial quantities from human uterine tissue and urine (Rijken *et al.*, 1979; White, Barlow & Mozen, 1966). The enzymes from these different sources are structurally and antigenically distinct proteins. Uterine tissue plasminogen activator (TPA) has a molecular weight of about 70,000 and is a fibrin-dependent enzyme (Hoylaerts *et al.*, 1982) whereas the urinary plasminogen activator, urokinase, is not dependent upon fibrin to enhance its action and displays a molecular weight of 54,000. It is of great interest to know which enzyme is involved in skin biochemistry and wound healing processes and towards this end we have partially purified the enzyme from fibroblasts and examined its fibrin binding and antigenic properties.

METHODS

Primary human fibroblasts were obtained from Flow Laboratory, Irving, U.K. (F7000) and cultured in Dulbecco's modification of Eagle's medium supplemented with 5–10% donor calf serum and buffered with 10 mmol/l HEPES. These cells were maintained as confluent monolayers; conditioned media were collected at regular intervals and stored at −30°C until used. All cell culture reagents and materials were purchased from Flow Laboratories, Irving, U.K.

Plasminogen activator was assayed as described by Walton (1966) and the assay calibrated with dilutions of a urokinase standard (Institut Choay, Paris, France).

Plasminogen activator was isolated from conditioned media by the method described by Rijken & Collen (1981). This method consists of successive chromatography on Sepharose zinc chelate, Sepharose concanavalin A and finally gel filtration on Sephacryl 300. All chromatography materials were purchased from Pharmacia Fine Chemicals, London, U.K. The Sephacryl 300 gel filtration column was calibrated with proteins of known molecular weights (IgG, 148,000; serum albumin, 68,000; ovalbumin, 43,000; soybean trypsin inhibitor, 21,500; and myoglobin, 18,500) purchased from Sigma Chemicals, Poole, U.K.

Human uterine tissue plasminogen activator (TPA) was prepared by the method of Rijken *et al.* (1979), and anti-TPA antibodies were raised in New Zealand White rabbits (J.M.Marshall & S.A.Cederholm-Williams, unpublished). Rabbit anti-TPA IgG fractions were prepared by chromatography on a column of Sepharose bound protein A.

Enzyme immuno-inhibition was carried out by mixing equal volumes of plasminogen activator preparations with anti-TPA or anti-urokinase IgG fraction, and measuring the remaining plasminogen activator activity.

Fibrin binding properties were examined by mixing fibroblast plasminogen activator (0·1 ml, 70 CTA units/ml) (CTA = Committee for Thrombolytic Activity) or urokinase (0·1 ml, 120 CTA units/ml) with human plasminogen free fibrinogen (1·0 ml, 3 mg/ml) (KabiVitrum, London, U.K.) and clotting the mixture by the addition of bovine thrombin (0·1 ml, 5 units/ml) (Diagen Diagnostic, Thame, U.K.). After 10 min incubation at 22°C the resulting clots were wound on to glass rods, washed twice in 5 ml volumes of 0·15 M NaCl and dissolved in 5 M urea (1 ml). The plasminogen activator activity of each fraction was measured.

All other reagents were purchased from Sigma Chemicals, Poole, U.K.

RESULTS

The conditioned media collected from confluent layers of fibroblasts regularly contained between 2–4 CTA units/ml plasminogen activator activity.

Conditioned media were thawed at 22°C and applied to a Sepharose zinc chelate column (4·4 × 13 cm) at 100 ml/h at 4°C. The column was washed free of non-bound proteins with 0·05 M Tris, 1·0 M NaCl, 1 g/l Tween buffer, pH 7·4 and the bound protein eluted by the application of 0·05 M imidazole, 0·05 M Tris, 1·0 M NaCl, 1 g/l Tween buffer, pH 7·4. Fractions (10 ml) were collected and the absorbance (E_{280nm}) and plasminogen activator content of each fraction measured (Fig. 1).

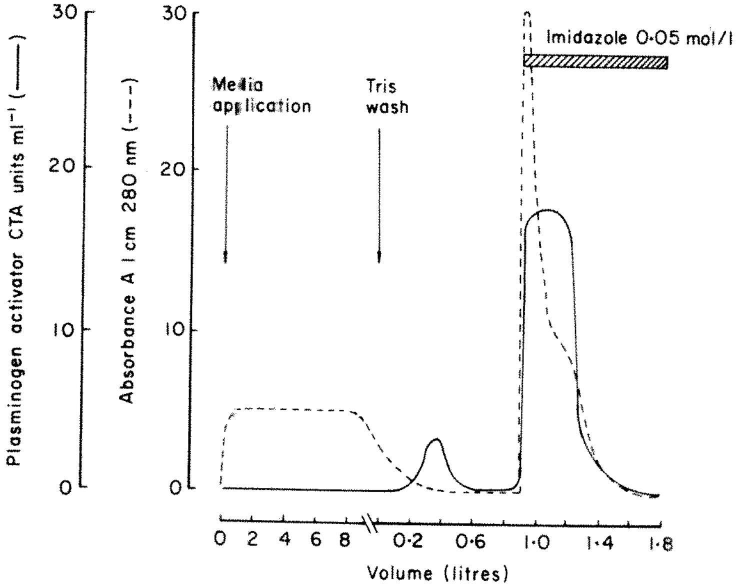

FIGURE 1. The application of fibroblast conditioned media to a column of Sepharose zinc chelate and the elution of the plasminogen activator by the application of imidazole.

Fractions containing plasminogen activator were applied to a column (2·6 × 19 cm) of Sepharose bound concanavalin A, which was washed free of unbound protein with 0·05 M Tris, 1·0 M NaCl, 1 g/l Tween buffer, pH 7·4 followed by 0·06 M α-D-methylmannoside in the washing buffer. The plasminogen activator was displaced from the column by a linear gradient of α-D-methylmannoside (0·06–0·6 mol/l) dissolved in 0·05 M Tris, 1·0 M NaCl, 1 g/l Tween buffer, pH 7·4. Fractions (10 ml) were screened for absorbance (E_{280nm}) and plasminogen activator activity (Fig. 2). Plasminogen activator rich fractions were concentrated by dialysis against polyethylene glycol (mol. wt. 2000) and made 1·6 mol/l with respect to KCNS by dissolving solid crystals into the concentrate.

The plasminogen activator concentrate was chromatographed on a column (1·6 × 90 cm) of Sephacryl 300 eluted with 1·6 M KCNS, 0·05 M Tris, 1·0 M NaCl, 1 g/l Tween buffer, pH 7·4 and fractions collected. Protein content of each fraction was determined by the method of Bradford (1976) and the plasminogen activator measured (Fig. 3).

The plasminogen activator eluted with a volume consistent with a molecular weight of 68,000. Fractions containing the enzyme were dialysed extensively against 0·05 M Tris, 1·0 M NaCl, 1 g/l Tween buffer, pH 7·4 to remove all traces of KCNS. The enzyme recovery is shown in Table 1.

The fibrin binding properties of the semipurified fibroblast plasminogen activator was assessed as described under methods. Virtually all (93%) of the enzyme bound to the fibrin clot, and it could be recovered (86%) from the redissolved clot. This contrasted with urokinase which showed only 12·5% of the enzyme to be associated with the redissolved clot (Table 2).

Enzyme immuno-inhibition: when the fibroblast plasminogen activator (5 CTA units/ml) was mixed with an equal volume of anti-urokinase IgG (which was capable of inhibiting 100 CTA unit/ml urokinase) no inhibition was obtained. Conversely, when mixed with anti-TPA IgG fraction (which was capable of inhibiting 30 CTA units/ml uterine tissue plasminogen activator) complete inhibition of the fibroblast enzyme was obtained.

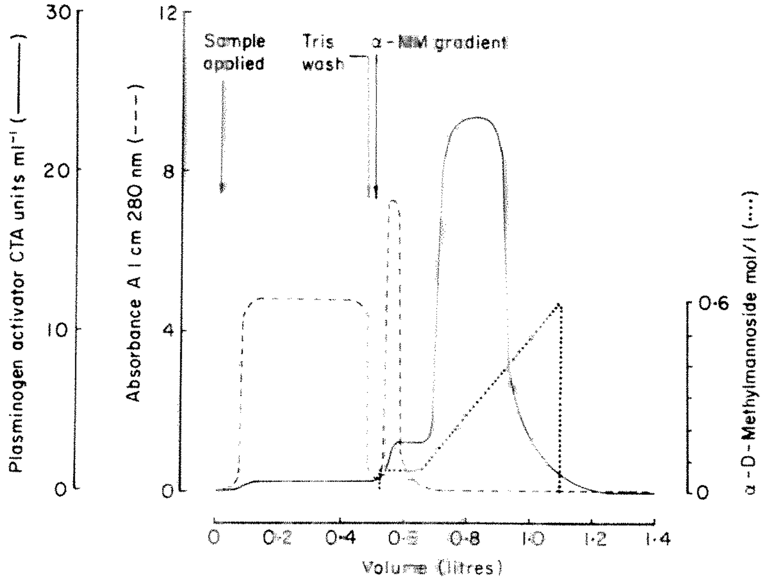

FIGURE 2. The application of the zinc chelate fraction to a column of Sepharose bound concanavalin A and the elution of the plasminogen activator by the application of a gradient of alpha-D-methyl mannoside (α-MM).

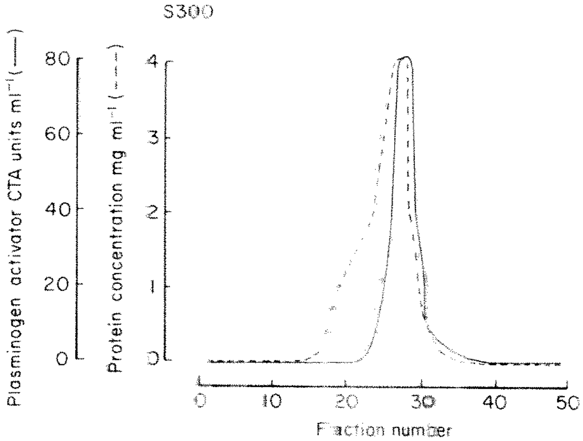

FIGURE 3. The gel filtration profile (Sephacryl 300) of the concentrate of the fractions recovered from the concanavalin A column. The plasminogen activator eluted with a volume coincident with serum albumen.

Fig. 4 shows that the fibroblast plasminogen activator is inhibited by anti-TPA IgG in a dose-dependent fashion, but urokinase is unaffected by this antibody. It is therefore concluded that the fibroblast plasminogen activator is antigenically identical to uterine tissue plasminogen activator and not urokinase.

Preliminary experiments with plasminogen activator-containing conditioned media from cultured keratinocytes and from cultured malignant squamous cell carcinoma (provided by S.Houlbrook, of this department) showed that in both cases the enzymes were completely inhibited by anti-TPA IgG and unaffected by anti-urokinase IgG.

TABLE 1. Showing the balance of recovered activity of fibroblast plasminogen activator following sequential chromatography

	Volume (ml)	Total activity (CTA units)	Total protein (mg)	Recovered activity (%)
Conditioned media	8000	2800	40,000	100
Zinc chelate eluate	500	2500	7000	89
Concanavalin A eluate concentrate	4	800	123	28·5
Sephacryl 300 fractions 26–30	30	400	30	14·4

TABLE 2. Showing the fibrin binding properties of fibroblast plasminogen activator (PA) and urokinase. Each value is the mean of three determinations

	CTA units added	Units remaining in clot supernatant	Units in wash 1	Units in wash 2	Units recovered from the clot
Fibroblast PA	7·0 (100%)	0·5 (7%)	0	0	6·0 (86%)
Urokinase	12·0 (100%)	7·0 (58%)	2·5 (20·8%)	0·9 (7·5%)	1·5 (12·5%)

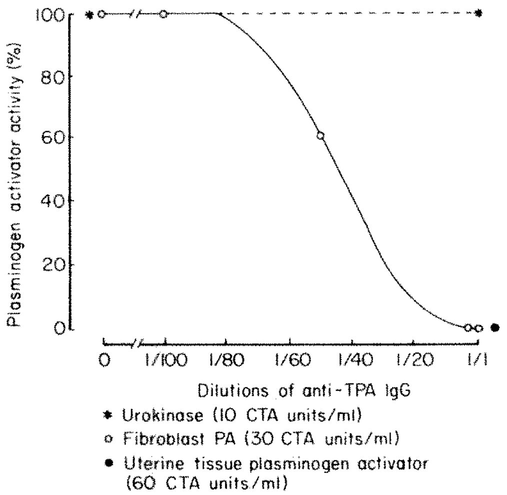

FIGURE 4. The enzyme immuno-inhibition of fibroblast plasminogen activator with varying dilutions of anti-TPA IgG fraction.

DISCUSSION

It is clear that plasminogen activator secreted by human fibroblasts in culture is the fibrin binding tissue plasminogen activator (mol. wt. 68,000) and not urokinase. Though the enzymes from cultured keratinocytes and malignant squamous cells have not yet been isolated, the antibody inhibition data suggest that these enzymes may also be tissue plasminogen activator and not urokinase.

If TPA is the enzyme responsible for generating the proteolytic activity in skin then a proper regard must also be given to the role of fibrin since it catalyses the action of this enzyme (Hoylaerts *et al.*, 1982). Fibrin also protects plasmin from the inhibitory action of alpha-2-antiplasmin (Wiman, Lijnen & Collen, 1979) and may therefore play a significant role in the proteolytic biochemistry of skin remodelling.

Work is currently under way to purify plasminogen activator from squamous cells and to develop an antibody which is good enough to carry out immunohistological studies on skin.

ACKNOWLEDGMENTS

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The dermal lymphatics in lymphoedema visualized by indirect lymphography

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SUMMARY

Iotasul®, a new water-soluble contrast medium, is very suitable for subepidermal infusion (indirect lymphography) for the demonstration of small (initial) dermal lymphatics in the macro-range (precollectors). By this method normal lymph vessels can be demonstrated in the healthy skin of hands and feet which are so fine that they cannot be punctured for direct lymphography.

We studied thirty-eight patients with various forms of lymphoedema by indirect lymphography. In patients with congenital lymphoedema (particularly Milroy's disease) the initial dermal lymphatics in the lymphoedematous region were absent but large lymph collectors were present. Hyperplasia of dermal lymphatics in the form of an extremely fine net was seen in most patients with lymphoedema praecox and tardum.

In some forms of primary lymphoedema the defect may be in the initial dermal lymphatics. In secondary lymphoedema, the changes in the peripheral lymphatics (mainly distension and congestion) are apparently a consequence of the primary lesion in the lymph nodes and large collectors. Indirect lymphography might be useful for the early diagnosis of mild lymphoedema and for excluding patients with hypoplasia of initial lymph vessels from surgery.

In the past, small (initial) dermal lymph vessels could be evaluated radiologically only in the presence of valvular incompetence in the collectors. Direct lymphography, i.e. the injection of contrast material into an exposed lymph vessel, then leads to reflux into the periphery and, hence, also to staining of collectors and precollectors in the skin (dermal backflow).

The newly developed water-soluble contrast medium Iotasul® (Schering AG, Berlin) (Siefert *et al.*, 1980) now allows antegrade visualization of dermal lymphatics by means of subepidermal injection. This technique makes it possible for the first time to elucidate certain aspects of dermal lymph drainage in various skin diseases. This new information about dermal lymphatics requires the conventional classification of lymphoedema to be revised.

METHODS

Thirty-eight patients with lymphoedema, aged between 15 and 61 years, were studied. The

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diagnosis was made on the basis of the clinical picture, and any disturbance of lymph transport was verified by prefascial isotope lymphography (Lofferer, Mostbeck & Partsch, 1972) with 99 mTc-labelled sulphur colloid (Lymphoszint®, Solco, Basle).

Twenty-one patients (nineteen women, two men) had primary lymphoedema. Two had the congenital hereditary form of Milroy, three had the congenital non-hereditary form, nine had lymphoedema praecox (onset before age 35 y) and seven had lymphoedema tardum (onset after age 35 y).

Seventeen patients (eleven women, six men) displayed secondary lymphoedema (ten following surgery for malignant tumours and subsequent irradiation, five after recurrent erysipelas and two after trauma).

The results of direct lymphography and histological examination of the skin were available for some of the patients. Twenty-seven extremities without clinical lymphoedema (contralateral side of lymphoedema patients or patients with other diseases) were studied as normal controls.

Technique

Iotasul®, a non-ionic water-soluble dimeric contrast medium was infused intracutaneously by means of a motorized pump (0·1–0·15 ml/min), usually at four different sites (2·5–5 ml per site). Subepidermal puncture with thin 'butterfly' needles was essential (Partsch, Wenzel-Hora & Urbanek, 1983).

The preferred injection sites are the dorsal folds of the toes and fingers, the backs of the hands and feet, and the tip of the fingers and toes. Symmetrical sites on the diseased and healthy extremities are usually examined.

Infusion of the contrast material causes a brief burning sensation at the start, while local reddening of the skin has so far been observed in only one of sixty-five patients studied. No general side-effects have been seen.

RESULTS

Healthy extremity (normal)

Thin lymphatics begin to fill from the spreading contrast medium depot just a few minutes after the start of the infusion. Some collectors are demonstrated that are so fine as to be inaccessible to puncture for direct lymphography (Fig. 1a).

Congenital hereditary lymphoedema (Milroy syndrome)

Two patients failed to exhibit any lymphatics on injection of the contrast medium into the lymphoedematous skin of the legs. In both cases, the examination revealed only cuff-shaped structures apparently corresponding to the diffusion of the contrast material along the adventitia of blood vessels (Fig. 2a).

Surprisingly, one of the two female patients displayed large, tortuous, pathological lymph collectors with extravasation on the back of the foot on injection of the contrast medium into the tip of a toe (Fig. 2b). Direct lymphography in this patient some years previously had failed. Because of the absence of lymph vessels even on biopsy the patient had been classified as aplasia.

Congenital non-hereditary lymphoedema

Two out of three women diplayed hyperplasia of precollectors and collectors. In one case, dilated lymph vessels in which the contrast medium was moving could be seen with the naked eye under the epidermis of the medial edge of the foot.

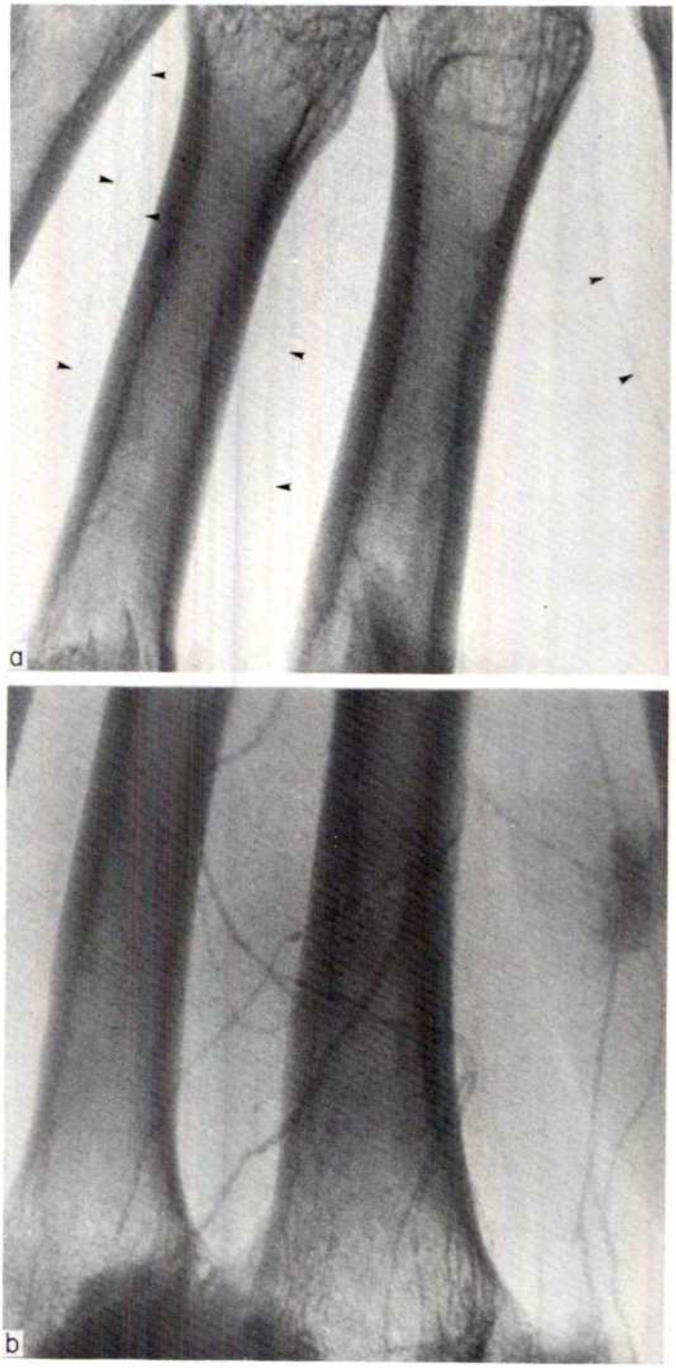

FIGURE 1. A 25-year-old normal woman, injection of Iotasul® into the interdigital skin of the hands. (a) Normal lymph collectors on the back of the hand (arrowheads) (diameter less than 0·1 mm). (b) Congested lymph collectors in artificial lymphoedema consequent to repeated self-application of a tourniquet on the upper arm.

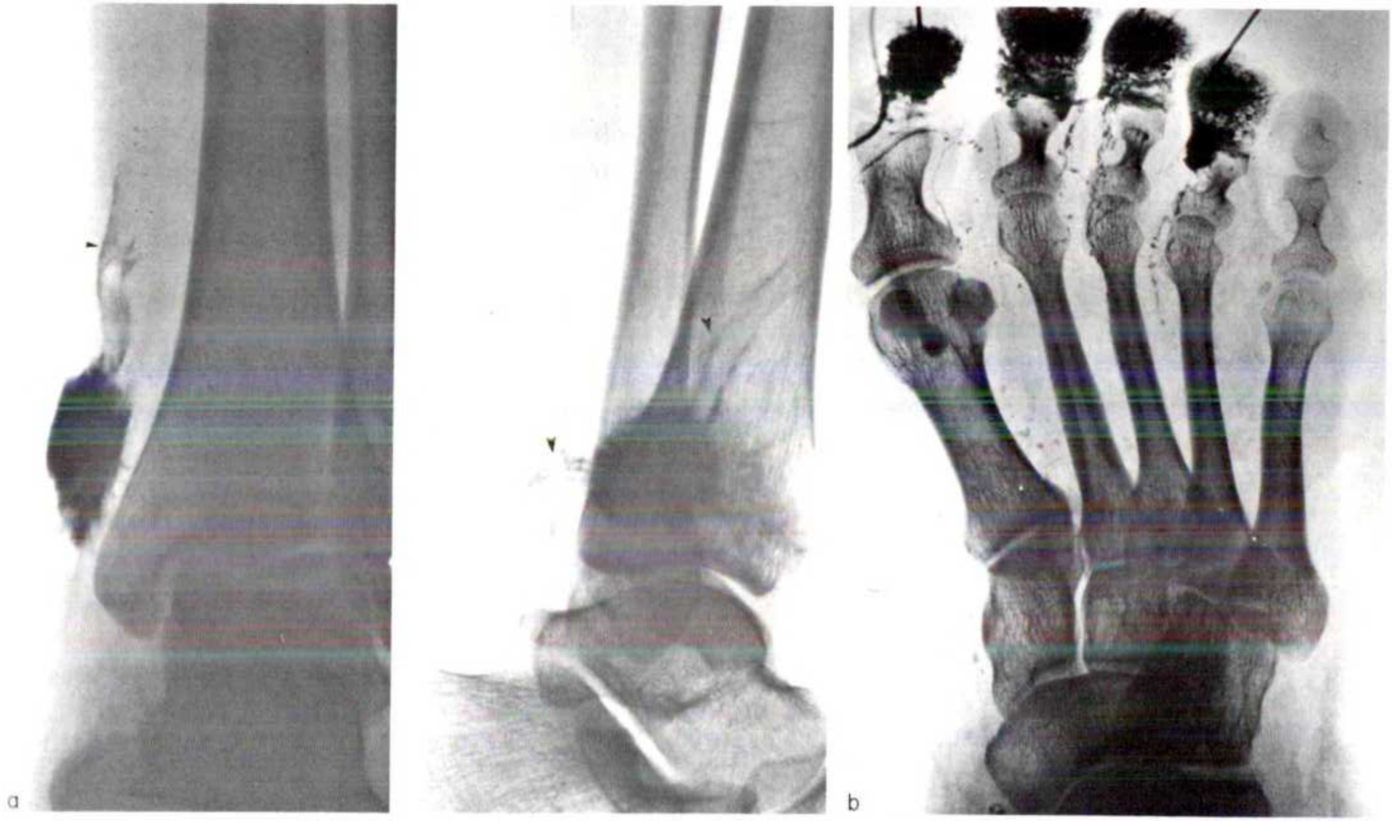

FIGURE 2. (a) A 22-year-old woman with congenital hereditary lymphoedema (Milroy's disease). An intracutaneous contrast medium depot at the inner ankle bone displays a strikingly homogeneous configuration. Instead of efferent lymphatic pathways, structures become opacified which apparently correspond to diffusion in the adventitia of blood vessels. (b) After infusion into the tips of the toes, demonstration of dilated, pathological collectors and extravasation in the region of the lymphoedema. The findings are indicative of hypoplasia or aplasia of initial lymphatics (2a) with conserved collectors (2b).

In the third case, injection of the contrast medium into the non-oedematous skin over the middle phalanx of the finger revealed lymph vessels which were also visible in a proximal direction in the region of the massive lymphoedema. In contrast, infusion into the lymphoedematous skin at the proximal phalanx led only to visualization of 'periadvential cuffs', but no lymph vessels. The situation therefore corresponds to that in the woman with the Milroy syndrome described above.

Lymphoedema praecox and lymphoedema tardum

Hyperplasia of the dermal precollectors in the form of extremely fine pathological reticular structures was the commonest finding in these patients, while few large collectors were demonstrated in most cases (Fig. 3).

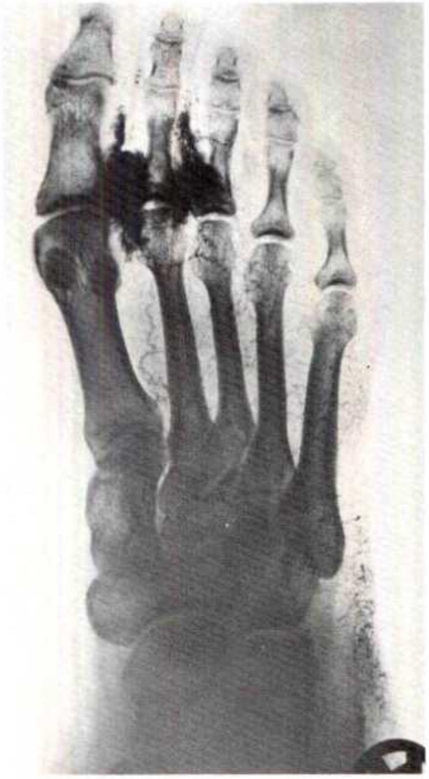

FIGURE 3. A 27-year-old woman with lymphoedema praecox. Dense nets of pathological precollectors and collectors with dermal backflow in the toes. Two years previously, after a failed attempt at direct lymphography, she was classified as hypoplasia.

In three patients with lymphoedema praecox, contrast medium drainage took place via deep collectors in the region of the foot and distal lower leg (Fig. 4).

Secondary lymphoedema

The commonest finding in these patients was an increase and thickening of precollectors and collectors (Fig. 1b).

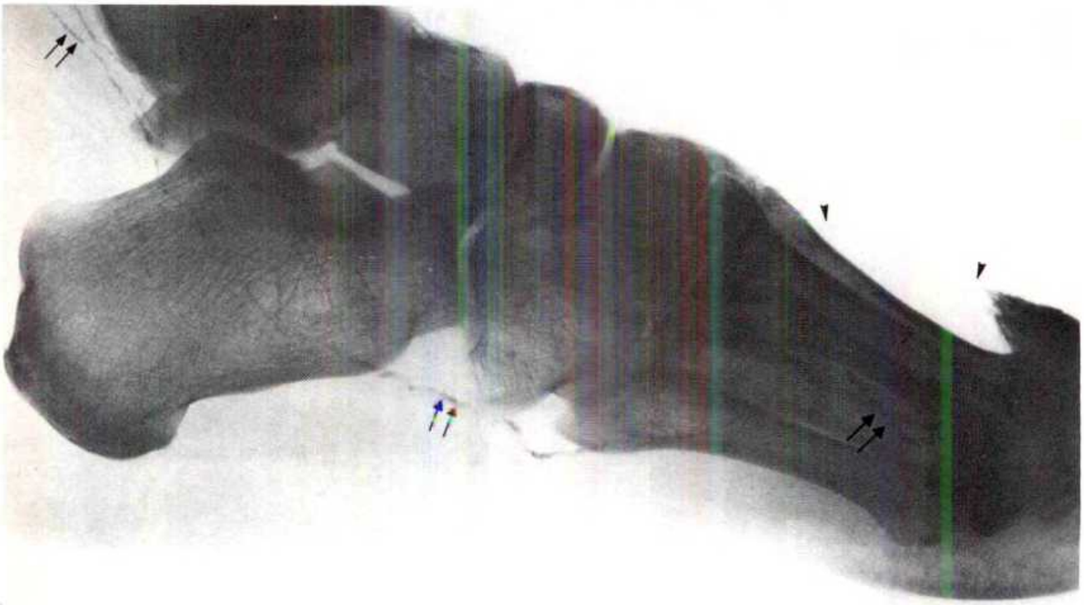

FIGURE 4. A 21-year-old woman with lymphoedema praecox. After infusion of the contrast medium in the region of the interdigital folds of the feet, visualisation of extremely fine lymphatics on the back of the foot (▼) and demonstration of deep, wide-calibre lymph collectors (↑↑).

TABLE 1. Evaluation by indirect lymphography (Iotasul®)

Form of lymphoedema	*n*	Precollectors			Collectors		
		Hyperplasia	Hypoplasia	Uncertain	Hyperplasia	Hypoplasia	Uncertain
Primary lymphoedema							
Milroy	2	—	2	—	1	—	1
Congenital non-hereditary lymphoedema	3	2	1	—	1	1	1
Lymphoedema praecox	9	8	1	—	2	6	1
Lymphoedema tardum	7	3	2	2	—	5	2
Secondary lymphoedema	17	13	2	2	14	3	—
Total	38	26	8	4	18	15	5

Table 1 summarizes the partial discrepancy between findings in the dermal precollectors and the larger collectors in the individual forms of lymphoedema.

DISCUSSION

Experience with direct lymphography shows that hypoplasia of the large collectors predominates in the primary forms of lymphoedema, and hyperplasia in the secondary forms (Kinmonth, 1982). Dilatation and congestion of the lymphatics in secondary lymphoedema can be explained by a proximal drainage obstruction, particularly in the lymph nodes.

Previously, little was known about the configuration of the small dermal lymphatics. Then, using fluorescence microlymphography, Bollinger *et al.* (1981) visualized lymph capillaries in the skin. The studies showed that lymph capillaries are completely absent only in congenital hereditary lymphoedema (Bollinger *et al.*, 1983) while the capillary nets in all other forms of lymphoedema are usually more extensive and of a wider calibre than in healthy subjects. Only the most superficial lymph structures immediately under the epidermis can be evaluated with the fluorescence technique.

Indirect lymphography with Iotasul® closes the gap between microlymphography and direct lymphangiography by visualizing the lymphatic pathways between the capillaries and the large collectors. In certain regions the contrast material diffuses immediately after the start of infusion in the form of a reticular structure resembling the capillary nets of microlymphangiography.

In healthy skin, the lymph collectors are so thin that they are frequently not demonstrable by direct lymphography without the use of stasis. Thus, the method introduced here makes possible for the first time the routine *in vivo* radiological demonstration of normal lymph collectors on the backs of the hands and feet and on the fingers and toes without the need for artificial manipulation.

The Milroy syndrome is characterized by the absence of initial lymphatics in the lymphoedematous skin, but lymph collectors which can transport lymph proximally from distal, clinically normal sections of extremities without any impairment of function are present.

Together with the findings of Bollinger *et al.* (1983) a form of lymphoedema can therefore be established which is caused by hypoplasia or aplasia of the initial dermal lymphatics. In our series, the two women with a Milroy syndrome and one woman with congenital non-hereditary lymphoedema can be classified in this way. In these cases, attempts at surgical reconstruction and lymphovenous anastomosis surgery are doomed to failure because of the lack of a functional initial lymphatic network.

Most types of lymphoedema praecox and tardum are associated with hyperplasia of dermal precollectors in the form of fine reticular structures.

The observation that hypoplasia of dermal lymphatics occurred in some patients, particularly those with lymphoedema tardum, might be explained in three ways:

(1) Some of the patients examined already displayed toughening of the skin, which made the examination technically difficult.

(2) A dying-back mechanism of originally dilated precollectors with thrombosis is conceivable in long-standing cases (Fyfe, Wolfe & Kinmonth, 1982).

(3) Lymph vessels can be occluded from the outside by tough, fibrotic connective tissue.

It must in any case be emphasized that a striking discrepancy exists in lymphoedema precox and lymphoedema tardum between the predominant hyperplasia of dermal precollectors, analogous to the changes in the region of the lymph capillaries (Bollinger *et al.*, 1983), and the simultaneous hypoplasia of collectors. We believe that hyperplasia of the initial lymphatic nets can be regarded as a consequence of hypoplasia in the large collectors or lymph nodes with consequent distal stasis.

Lymphangioma cysts, highly suitable as puncture sites for indirect lymphography, may even develop in extreme cases (as in secondary lymphoedema) (Whimster, 1976). The possibility of a primary anatomical and functional lesion of the initial lymphatics with consequent proximal changes cannot, however, be reliably ruled out in the individual case.

In most cases, secondary lymphoedema is characterized by dilatation and congestion of collectors and precollectors. The films from indirect lymphography are then very much the same as those obtained with the direct technique, in which the dermal lymphatics may also opacify because of the dermal backflow mechanism.

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Trichothiodystrophy: an electron-histochemical study of the hair shaft

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SUMMARY

We have used sensitive electron-histochemical methods to study the subtle ultrastructural variations of cystine incorporation into the hair shaft in trichothiodystrophy.

We have shown a general reduction in the cystine (sulphur) content of both the cuticle and the cortex. Discontinuity, and in some cases, complete absence of the cuticular A-layer results in premature weathering of the cuticle and weakening of the hair shaft. The ultrastructural findings support the work of Gillespie & Marshall (1981) in demonstrating the absence or re-characterization of the high sulphur matrix proteins and show further evidence for the incorporation, and abnormal distribution and deposition of sulphur-rich proteins in the hair cortex and cuticle.

We conclude that the similar yet different results obtained from each patient's hair sample are characteristic of trichothiodystrophy, a neuro-ectodermal symptom complex which may represent a final common pathway of more than one metabolic disturbance.

Trichothiodystrophy, described by Price *et al.* (1980), is characterized by highly specific clinical, light microscopic and biochemical abnormalities of the hair shaft in patients with severe congenital neuro-ectodermal changes (Pollitt, Jenner & Davis, 1968), which may include mental and physical retardation, lamellar ichthyosis, nail dystrophy, ocular dysplasia, dental caries and reduced fertility.

The hair is typically very short, brittle and flattened with both trichorrhexis nodosa-like and trichoschisis fractures, and shows advanced weathering of the cuticle. The hair usually shows alternating bright and dark bands when viewed by plane-polarized transmitted light. Biochemically, the hair is low in sulphur and two-dimensional electrophoresis shows a reduced high-sulphur matrix fraction (Gillespie & Marshall, 1981). It is reasonable to postulate that such biochemical and physical abnormalities of the hair shaft relate to a decrease in the sulphur-rich A-layer and the exocuticle of the cuticle, and the cystine-rich globular matrix proteins of the hair cortex. In order to define accurately the anatomical site of these biochemical changes, we have studied the hair of four affected children using a sensitive electron-histochemical technique to study the distribution of cystine (sulphur) in transverse sections of the hair shaft (Swift, 1968; Gummer, Dawber & Swift, 1981).

METHODS

Scalp hair samples from four cases of trichothiodystrophy, diagnosed by clinical examination and light and polarizing microscopy, were embedded in Spurr's low viscosity embedding media (Spurr, 1969). Ultrathin sections of silver-grey interference colours were cut using a diamond knife on an LKB III Ultratome. Eight to ten hairs from each patient were sectioned at the proximal and distal ends of the hair shaft. The sections were collected on 400 mesh gold grids and stained as follows:

(a) Ammoniacal silver nitrate (Swift, 1968), used as a cystine-specific positive stain.
(b) Uranyl acetate and lead citrate (Reynolds, 1963), used as a general morphological stain and a cystine-negative stain when supported by ammoniacal silver nitrate.

All sections were viewed on a Seimens Elmiskop 101 transmission electron microscope.

Patient 1
Dr V.H.Price, California (Price *et al.*, 1980). Cross-sectional appearance—oval.

Cuticle. Ammoniacal silver nitrate. There was a general reduction in the number of cell layers towards the distal end of the hair shaft. The number of cell layers at the proximal end of the hair shafts varied from 4 to 7 with a reduction in number at the ends of the oval cross-section.

The A-layer and inner layer were either negative or showed a greatly reduced uptake of stain (Fig. 1). The cell membranes showed continuous undulations and occasional large radial 'folds'. Pockets of cystine-positive material were seen within the endocuticle.

Cuticle. Uranyl acetate and lead citrate. Both the A-layer and the inner layer stained positive with this method (Fig. 2). The boundary between the endocuticle and the exocuticle was poorly defined, with large pockets of negatively stained material occurring within the endocuticle. An extensive endocuticular ultrastructure was seen which comprised membrane-like and membrane-bound inclusions within amorphous ground substance (Fig. 2).

Cortex. Ammoniacal silver nitrate. Due to the poor uptake of the stain despite increased staining times it was not possible to distinguish the ultrastructure of the cortical cells. All the cortical cells showed a uniformly low uptake of stain.

Cortex. Uranyl acetate and lead citrate. Many cortical cells showed well-defined macrofibrils with a microfibrillar ultrastructure. These were considered to be orthocortical cells. Others showed a dense, uniform, microfibrillar, matrix composition and were considered to represent paracortex. No well-defined macrofibrils were seen in these cells.

Both pigment granules and the cortical cell membranes appeared to be normal.

Patient 2
Cross-sectional appearance—oval.

Cuticle. Ammoniacal silver nitrate. The stain intensity was, in the majority of sections studied, comparable to normal hair. Numerous cell layers, up to seven, were seen in all but the distal ends of the hair shafts. There was, however, a reduction in the numbers of cell layers at the ends of the oval cross-section. The cell membranes showed marked undulations with some protrusions of

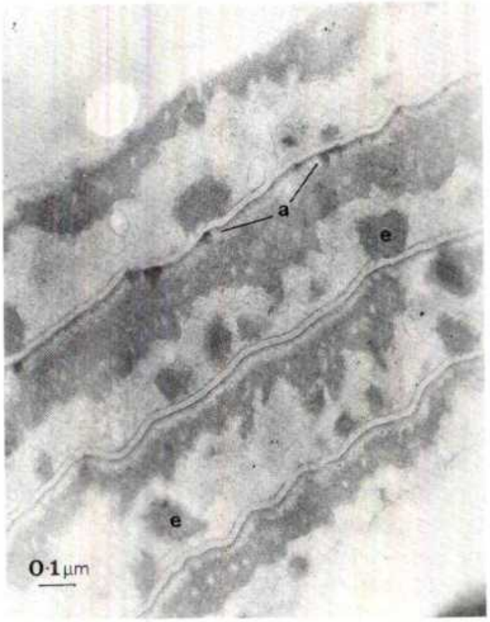

FIGURE 1. Transverse section of the hair cuticle (patient 1). The overall stain intensity is reduced. The A-layer (a) shows only slight positive staining. Abnormal cystine-positive inclusions are present within the endocuticle (e). Stain: ammoniacal silver nitrate.

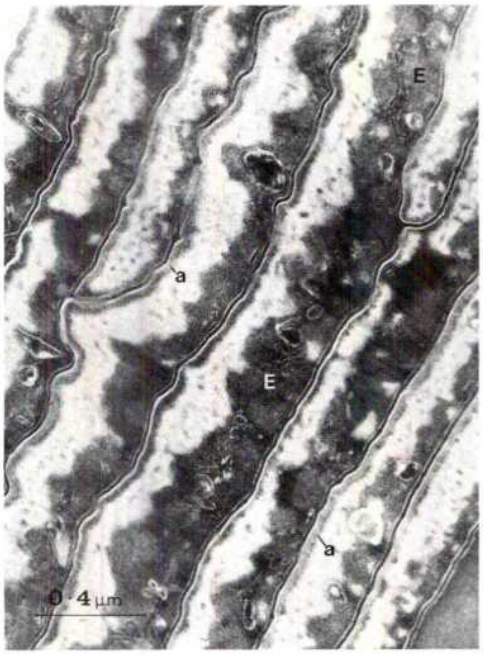

FIGURE 2. Transverse section of the hair cuticle (patient 1). Abnormal positive staining of the A-layer is seen (a). The endocuticle shows an extensive ultrastructure (E). Stain: uranyl acetate/lead citrate.

the membrane complex into the cell mass. Many of the cuticle cells showed a normal lamellar substructure of outer A-layer, exocuticle, endocuticle, and inner layer, whereas others showed wide variations, particularly of the A-layer, in width and stain intensity both within individual cells and in comparison with adjacent cells. In some cells the A-layer did not stain (Fig. 3).

Cuticle. Uranyl acetate and lead citrate. In those cells where the A-layer was negative with silver staining then positive staining was seen with this technique (Fig. 4). Where silver staining was reduced then slight positive staining was encountered. The inner layer of each cell varied similarly. The boundary between the endocuticle and the exocuticle was not well defined with numerous pockets of cystine-rich material within the endocuticle. The endocuticle was seen to have a detailed ultrastructure of membrane-like and membrane-bound inclusions.

Cortex. Ammoniacal silver nitrate. The cells of the cortex were divided into two populations according to the degree of stain uptake (Fig. 5): (a) those cells showing a stain intensity comparable to normal hair, and (b) those cells showing a stain intensity less than that of normal hair. Numerous pockets of cystine-rich material of varying shape and size were seen though only within the cells of group (a). No microfibrils were seen within the cystine-rich material though they were well defined at the periphery of the inclusions (Fig. 6). Overall the cells of group (a) showed a well-defined macrofibrillar substructure, each composed of microfibrils, often in pseudohexagonal array, embedded in a cystine-rich matrix. In many of these cells there appeared to be an increased macrofibrillar spacing by negatively stained material. A detailed ultrastructure was often difficult to observe in the cells of group (b) due to the low uptake of metallic silver which reduced overall section contrast.

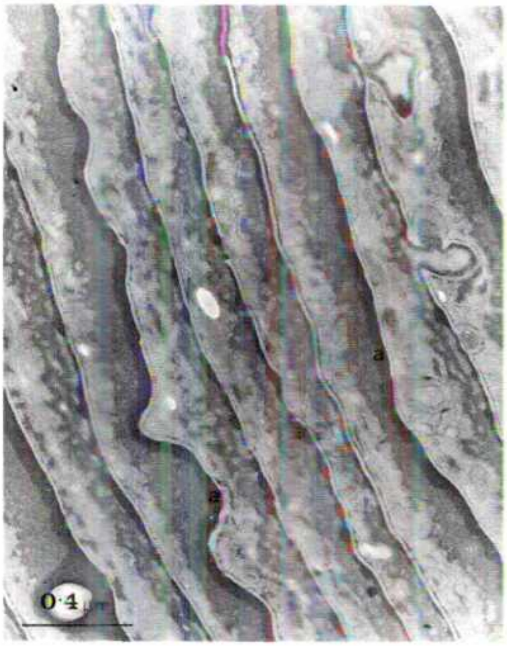

FIGURE 3. Transverse section of the hair cuticle (patient 2). The A-layer (a) is often discontinuous and shows marked variations in width. Stain: ammoniacal silver nitrate.

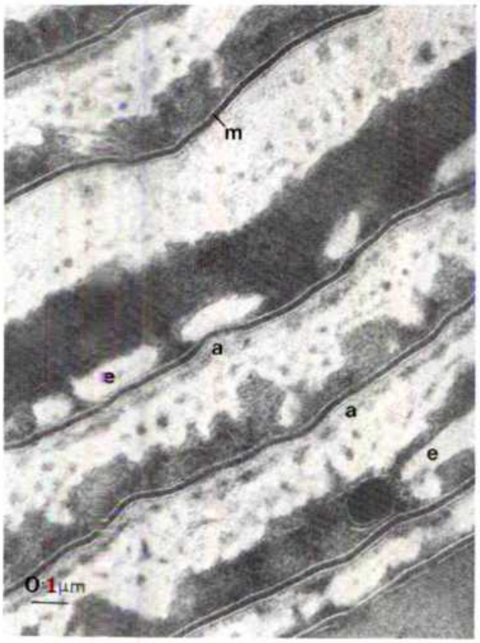

FIGURE 4. Transverse section of the hair cuticle (patient 2). The A-layer (a) shows light positive staining. Small areas of negatively stained 'sulphur-rich' material are seen within the endocuticle (e). m = cell membrane complex. Stain: uranyl acetate/lead citrate.

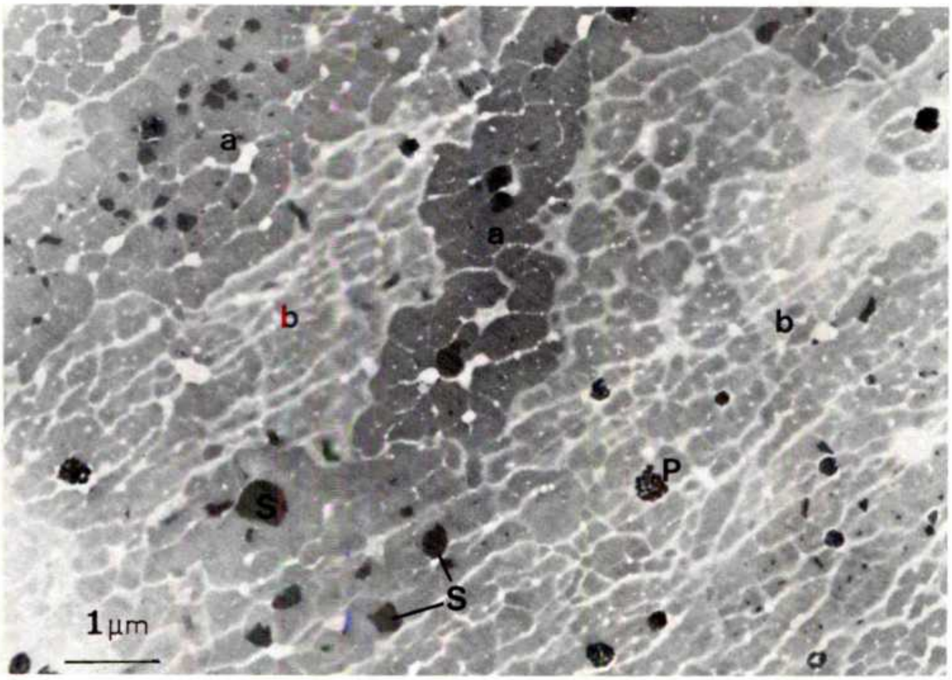

FIGURE 5. Transverse section of the hair cortex (patient 2). Two cell populations (a and b) are present, distinguishable by the degree of stain uptake. The cells of group (a) show large inclusions of sulphur-rich material (S). P = pigment. Stain: ammoniacal silver nitrate.

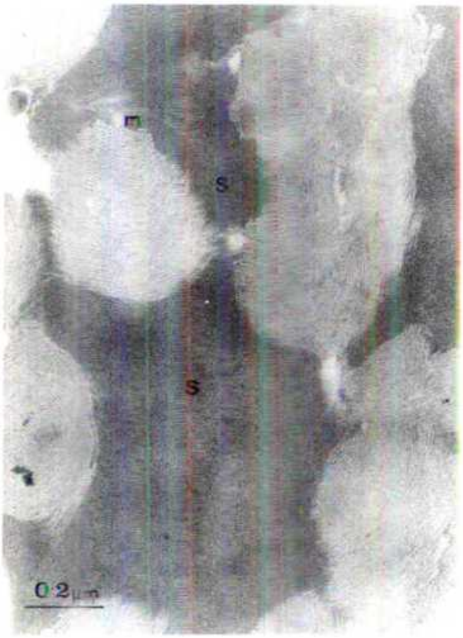

FIGURE 6. Transverse section of the hair cortex (patient 2). Large sulphur (cystine)-rich intermacrofibrillar inclusions were found (S) in the cortical cells of group (a). Well-defined microfibrils are seen at the edge of the inclusions (m). Stain: ammoniacal silver nitrate.

Cortex. Uranyl acetate and lead citrate. All the cells of the cortex stained with a uniform stain intensity. The cystine-positive inclusions encountered with silver staining did not stain with this method (Fig. 7). Cortical cell membranes, pigment granules, and nuclear remnants showed staining characteristics comparable to normal hair.

Patient 3
Dr V.H.Price, California. Cross-sectional appearance—elongated oval.

Cuticle. Ammoniacal silver nitrate. None of the cuticle cells viewed appeared normal. Only one cell was seen to have a continuous, positive A-layer. All other cells showed a clear, unstained region corresponding to the site of the A-layer. The exocuticle, present to varying degrees in both width and continuity, showed a decreased positive stain when compared with normal hair. In many cells the exocuticle was arranged in discrete blocks separated by a clear unstained zone. The endocuticle showed numerous inclusions of cystine-positive material, circular in cross-section and with intensely stained cores (Fig. 8). It was considered that these inclusions occurred within the endocuticles; however, as few cuticle cells had a well-defined lamellar substructure, there was doubt as to the true location of these structures. Towards the distal ends of the hair shafts there was a rapid increase in cuticular weathering; however, the cystine-positive inclusions showed no obvious signs of breakdown in these regions.

Cuticle. Uranyl acetate and lead citrate. The cystine-positive inclusions seen with ammoniacal silver nitrate stained positively with this regime though few showed a densely stained core.

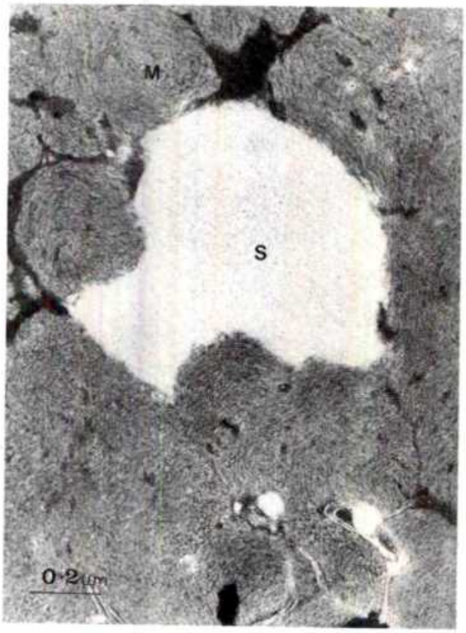

FIGURE 7. Transverse section of the hair cortex (patient 2). The cystine-positive inclusions of cell group (a) show negative staining with this technique (S). The macrofibrils show a well-defined ultrastructure (M). Stain: uranyl acetate/lead citrate.

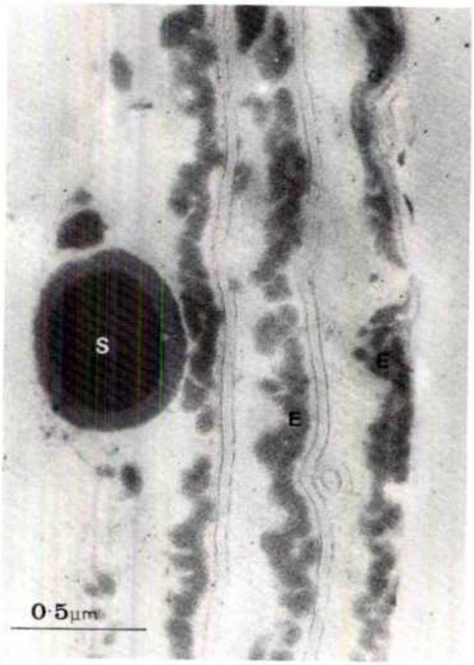

FIGURE 8. Transverse section of the hair cuticle (patient 3). The overall stain intensity is greatly reduced. The exocuticle shows discontinuous staining (E). The A-layer has not stained. Large cystine (sulphur)-positive, circular inclusions were often found within the endocuticle (S). Stain: ammoniacal silver nitrate.

Areas of cuticle cells without included bodies showed little or no division into a lamellar substructure. The cell membrane complex showed subtle undulations and varied in width continuously.

Cortex. Ammoniacal silver nitrate. In almost all of the sections viewed it was not possible to observe a cortical ultrastructure. Occasional cortical cells showed distinct macrofibrillar subunits composed of microfibrils embedded in a cystine-rich matrix, with large intermacrofibrillar cystine-positive inclusions (Fig. 9). Distinct microfibrils were rarely seen at the periphery of the inclusions.

Cortex. Uranyl acetate and lead citrate. All the cortical cells showed a well-defined ultrastructure. The intermacrofibrillar inclusions, positive with silver staining, did not appear to stain. Many cells showed distinct macrofibrillar subunits with a microfibrillar ultrastructure and were classed as orthocortical cells; other cells showed a uniformly dense distribution of microfibrills and were classed as paracortex. There was no specific distribution of the two cell types in the cross-sectional geography of the hair shaft. There appeared to be wide variations in the width of the intracellular cement of the cell membrane complexes.

Patient 4

Dr V.H.Price, California. Cross-sectional appearance—laterally flattened ovals.

Cuticle. Ammoniacal silver nitrate. There was a wide variation in the ultrastructural appearance of the individual cuticle cells. In general the A-layer did not stain. Some cells showed a discontinuous light positive stain of varying width at the site of the A-layer. The

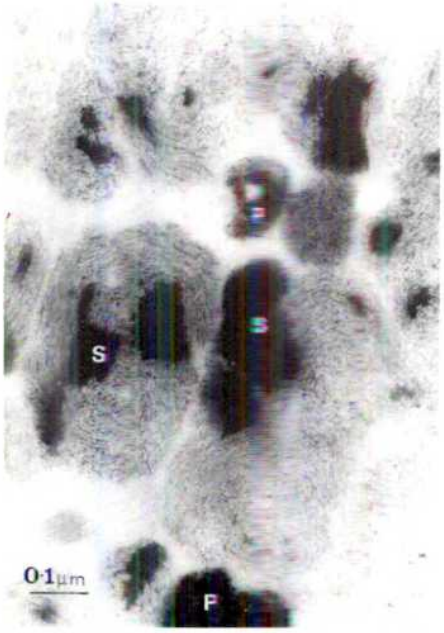

FIGURE 9. Transverse sections of the hair cortex (patient 3). Some cortical cells showed areas with distinct macrofibrils which often contained large cystine-positive inclusions (S). P = pigment. Stain: ammoniacal silver nitrate.

exocuticle showed similar variations in both stain intensity and width. Some cells showed the exocuticle to be lightly stained with local areas of increased stain intensity. The boundary between the exocuticle and the endocuticle showed some interdigitation. Some cells showed an exocuticle composed of block-like structures of cystine-positive material spaced by unstained zones. The endocuticle showed numerous areas of cystine-positive material.

Cuticle. Uranyl acetate and lead citrate. The A-layer appeared positively stained throughout its length including those areas that were positively stained with ammoniacal silvers. The endocuticle was seen to be positive with numerous membrane-like inclusions. All of the cells observed showed an endocuticular zone though it varied greatly in dimensions. The cell membrane complex showed marked undulations though large radial 'folds' were rarely seen.

Cortex. Ammoniacal silver nitrate. In all of the sections viewed the uptake of stain by the cortex was reduced when compared with normal hair. Some cells showed small pockets of intensely stained cystine-positive material with occasional, poorly defined microfibrils at the periphery of the inclusions. The majority of cortical cells showed a general light positive staining though no ultrastructure could be defined.

Cortex. Uranyl acetate and lead citrate. With this regime the cells were subdivided acording to general structure into orthocortex, with well-defined macrofibrils, and paracortex, with uniform ultrastructural staining and no well-defined macrofibrils. The cystine-positive areas observed with silver staining did not stain with this regime. The cortical membranes appeared as normal though there was wide variation in the width of the intracellular cement. Pigment granules appeared to be normal.

DISCUSSION

The ultrastructural and electron-histochemical findings from the hair shafts of four patients with trichothiodystrophy were all found to exhibit, to various degrees, the following changes:

(a) A general reduction in the stain intensity of both the hair cuticle and cortex when stained with ammoniacal silver nitrate, irrespective of increased stain concentration, prolonged staining times, and reduced washing periods.

(b) A focal abnormality of the cuticular A-layer which either failed to stain or stained as a discontinuous band of irregular thickness with ammoniacal silvers. With uranyl acetate and lead citrate the A-layer was abnormally positive.

(c) A generalized increase in the ultrastructural detail of the endocuticle, i.e. of membrane-like structures, when compared with normal hair cuticle cells.

(d) The presence of cystine-positive inclusions, as stained by ammoniacal silver nitrate, within the endocuticle of the cuticle cell and the macrofibrils of the cortex.

(e) The occurrence of two cortical cell populations, separable by their degree of silver uptake and not corresponding to the para- and orthocortical cell division as seen in normal hair.

The overall reduction in the stain intensity achieved with ammoniacal silver nitrate was predictable. Swift (1968) showed that ammoniacal silvers and silver methenamine could be considered as specific positive stains for sulphur as cystine and that there is almost certainly a positive stoichiometric correlation between the stain intensity and concentration of sulphur present. Amino acid analysis of trichothiodystrophic hair (Gillespie & Marshall, 1981) shows the hair to be particularly low in cystine—8·0%, compared with a normal 17·8% of cystine expressed as per cent amino acid residues, the reduction occurring in the ultra-high sulphur matrix proteins of the cortex, i.e. a fraction of those globular proteins interposed between the low sulphur microfibrillar proteins.

Advanced cuticular weathering along the total length of the hair shaft, as opposed to only at the distal end of normal fibres, is considered to be due to a focal abnormality of the A-layer. It is interesting to note that the A-layer, a region rich in cystine and consequently numerous high-energy disulphide bonds, occurs on the outer or environmental aspect of the cuticle cell. The positive staining encountered in the A-layer with uranyl acetate/lead citrate in trichothiodystrophic hair is normally reserved for the ionically and probably weaker endocuticle. Also the abnormal block-like arrangement seen in the A-layer is similar to that seen in the lower reaches of the normal follicle. As the cuticle cell undergoes keratinization, keratohyalin granules arranged adjacent to the outer cuticular plasma membrane gradually coalesce to form blocks and then a continuous A-layer and exocuticle. Such formative arrangements are not normally found in the upper parts of the follicle or in the hair, once it has passed the skin surface. The results appear to indicate that either there is a failure in the biochemical management of the oxidative process of keratinization of the cuticle, i.e. the absence of an enzyme, or the process of keratinization occurs but at a much reduced rate, such that by the time the keratinizing portion of the fibre reaches the upper half of the follicle it is either too far removed from the inductive and supply influences of the follicular bulb, or that dynamic forces pertinent to the lower parts of the follicle and probably essential for normal keratinization are no longer active. The end result of either hypothesis is, ultrastructurally, a 'partially' keratinized cuticle cell.

The increase in the endocuticular ultrastructure, far greater than normally observed, is again considered to be indicative of incomplete keratinization. The endocuticle of each cuticle cell is composed of effete cell organelles and cell cytoplasm. The layer is ionically bonded, as indicated by the intense positive staining with uranyl acetate and lead citrate, and is normally negative for cystine-positive material. The presence of numerous cytoplasmic remnants in the endocuticle of the final hair shaft suggests that normal keratinization, which occurs at the expense of the cell contents, is incomplete.

Partial formation of the A-layer and increased endocuticular detail must be indicative of a biochemical abnormality in the keratinization process simply because the wide variation in growth rates of hair in normal individuals, i.e. the speed at which the hair is produced, does not lead to ultrastructural abnormalities and weak hair. This is noteworthy in trichothiodystrophy in which growth is particularly slow, implying maximum follicular period in which the hair could complete keratinizaton.

The cortex of many of the hair shafts studied showed two populations of cortical cells. Normal hair contains both para- and orthocortical cells, identifiable by differences in arrangement, though both containing an easily identifiable microfibril/matrix composite. The two populations found in trichothiodystrophic hairs do not conform to the cells in normal hair. Due to the low silver uptake of many of the cortical cells and resulting low section contrast, it was often difficult to discern any appreciable ultrastructure. However, with uranyl acetate and lead citrate all of the cortical cells showed a similar stain uptake. The action of this stain regime on the cortex remains speculative as normal hair samples show a similar stain uptake. The presence of large deposits of cystine-positive material within the more intensely stained cortical cells could not be explained. Similarly, it is not known how the mechanical properties of the cortical cell, based on a fibre/matrix composite, are adversely affected by these localized deposits which do not contain the fibre component.

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The pathogenesis of amiodarone-induced pigmentation and photosensitivity

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SUMMARY

A new method has been used to measure tissue levels of amiodarone and its major metabolite desethylamiodarone. Amiodarone-pigmented skin has a drug and metabolite concentration ten times that of non-pigmented skin. Iodine-rich amiodarone and its metabolite have been detected in secondary lysosomes by energy dispersive analysis of X-rays. Amiodarone induces a phototoxic reaction with an action spectrum in both the UV-B and UV-A wavelengths.

Amiodarone is a highly effective anti-dysrhythmic agent which also has anti-anginal properties (Singh & Vaughan-Williams, 1970; Cote *et al.*, 1979; Marcus *et al.*, 1981). Photosensitivity is a common adverse reaction occurring in a high proportion of patients (Chalmers *et al.*, 1982; Harris *et al.*, 1983) and appears to be a dose-related phenomenon. A rarer problem is the occurrence in the light-exposed areas of blue-grey pigmentation.

We have investigated the pathogenesis of these adverse effects, employing a combination of biochemical and histopathological techniques. We employed selective high performance liquid chromatography to measure tissue levels of amiodarone (Am) and its major metabolite desethylamiodarone (DA). Furthermore, since Am and DA both contain iodine (Geerts, 1971), energy dispersive X-ray microanalysis (EDAX) has enabled us to detect the localization of Am and DA within the dermis (Slater *et al.*, 1983).

PATIENTS

Two male Caucasians, aged 44 and 52 years respectively, were referred with photosensitivity and pigmentation following chronic Am therapy. The first patient had taken 300 mg Am daily for 3 years, and was maintained on treatment during the investigations. The second had taken 800 mg daily for 2 years, but had stopped treatment 6 months before the tests were performed.

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METHODS

Monochromator studies were performed on non-exposed non-pigmented skin of the back (Magnus, 1976). Elliptical biopsies were taken from light-exposed pigmented areas on the forehead (Fig. 1) and covered non-pigmented areas on the abdomen. Samples of 20–100 mg were subjected to enzymatic digestion prior to analysis by high-performance liquid chromatography (Storey *et al.*, 1983).

Tissue was also examined by standard light microscopy using haematoxylin and eosin, and stains for lipofuscin (Sudan Black, Periodic acid–Schiff reagent, long Ziehl–Neelson method and thionin).

In addition to transmission electron microscopy (TEM), further analysis of the elemental nature of the tissue was obtained by EDAX. Unstained 100 μm sections were viewed with a Philips EM 400 and attached EDAX system linked to an EPIC computer and display system at a tilt angle of 30°, acceleration voltage of 60 kV and probe sizes of 200 and 500 nm.

RESULTS

Monochromator studies revealed abnormal acute responses at wavelengths of 307·5, 320 and 400 nm consistent with a phototoxic reaction to amiodarone.

Mean concentrations of Am and DA in light-exposed pigmented skin (179 and 826·5 mg/kg.

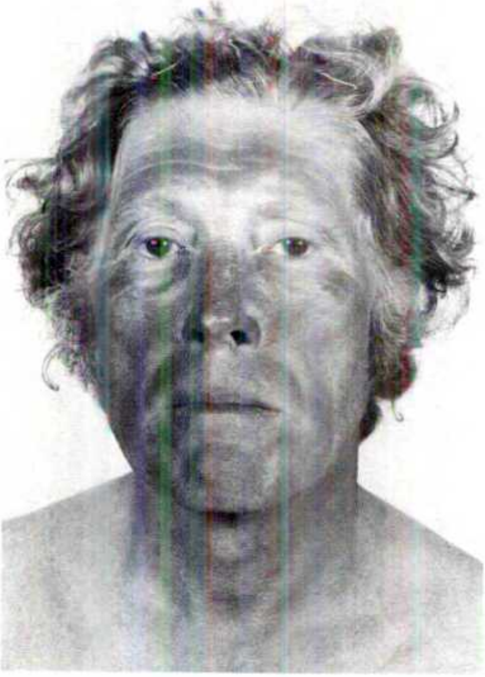

FIGURE 1. Amiodarone-induced pigmentation. Note that the pigmentation spared the hairline and periorbital regions.

wet weight) were ten times those in non-exposed areas (18·6 and 84 mg/kg). The respective plasma levels were 2·5 and 2·3 mg/l in one patient.

Sections of the light-exposed skin stained with haematoxylin and eosin revealed mid-dermal aggregates of yellow-brown granular pigment which was notably perivascular in distribution and localized in the cytoplasm of dermal macrophages. Special staining methods demonstrated that this pigment had the characteristics of lipofuscin (Fig. 2) (Matheis, 1972).

Electron microscopy revealed numerous electron-dense secondary lysosomes lying within dermal macrophages (Fig. 3). The lamellated myelin figure-like structures and associated vacuolation were characteristic. Many lysosomes contained lipofuscin which at lower magnification appeared amorphous (Fig. 4a) but could be shown at a higher magnification to have a paracrystalline structure. This was reminiscent of a fingerprint pattern, comprising varidirectional alternating parallel electron-dense and electron-lucent zones of 12 Å periodicity (Fig. 4b). This is a feature specific to amiodarone pigmentation. EDAX of light-exposed skin showed the presence of iodine, sulphur and iron within the electron-dense granules of the dermal macrophages (Fig. 5).

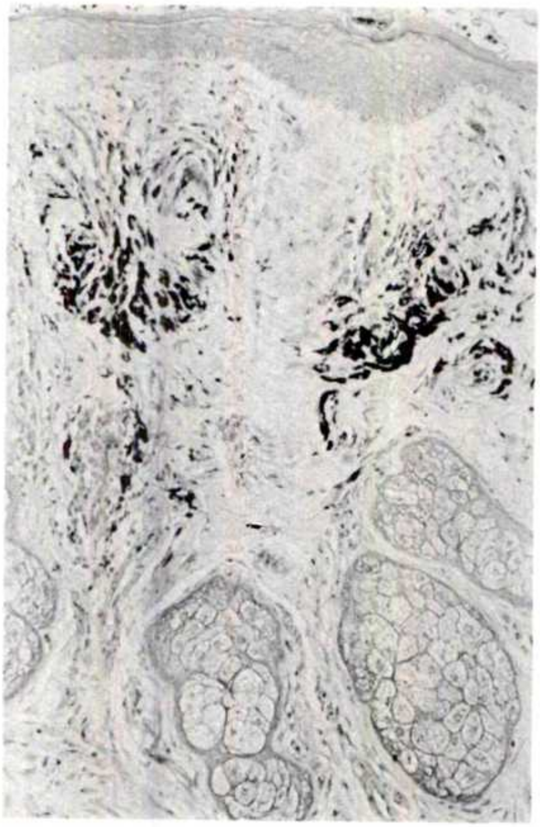

FIGURE 2. Positive Sudan Black stain in pigmented skin, suggesting the presence of lipofuscin within the dermal macrophages.

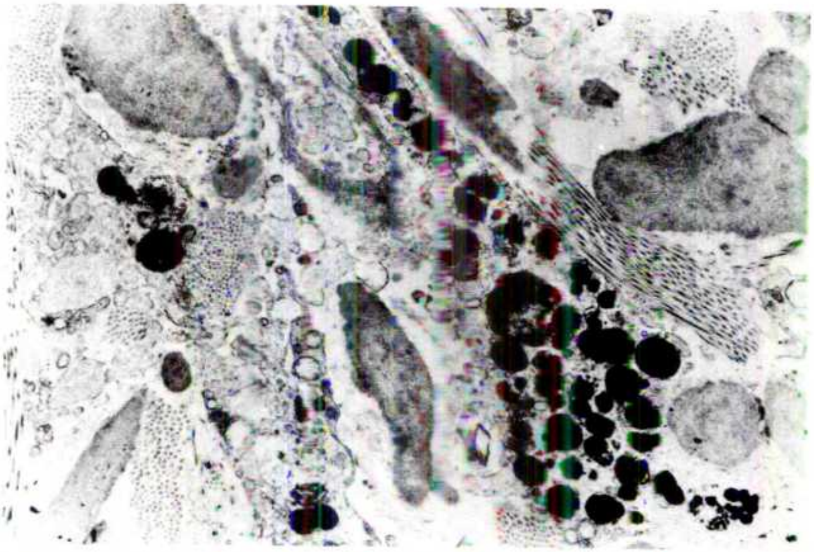

FIGURE 3. Numerous secondary lysosomes within dermal macrophages are filled with electron-dense material, TEM, ($\times$ 1950).

DISCUSSION

This study has demonstrated the presence of Am and its major metabolite DA in the dermis of patients receiving long-term therapy. Furthermore, ten times the concentrations of Am and DA were present in the light-exposed pigmented areas compared with the covered areas. We confirmed the presence of lipofuscin granules within the dermal macrophages shown previously by other researchers (Matheis, 1972). While studying tissue with the transmission electron microscope it has been possible to determine simultaneously its elemental composition by X-ray microanalysis. We have demonstrated a heavy concentration of iodine localized to the secondary lysosomal structures. Furthermore, the measured quantity of iodine correlated positively with the degree of electron density seen by electron microscopy. The deposited iodine is almost certainly derived from Am, a 600 mg daily dose of which contains 200 mg of iodine (Geerts, 1971). The monochromator light tests indicate a phototoxic reaction in both the UV-B and UV-A wavelengths. These results correlate well with the absorption spectrum of Am (Ferguson & Frain-Bell, personal communication). It is likely that UVR exposure induces Am and DA cell membrane binding both within the vessel wall and in the perivascular tissue. In addition the associated vasodilatation may allow increased diffusion of Am and DA to the perivascular tissues, resulting in the chronic accumulation of the drug and its metabolites.

Our finding of iodine bound to lipofuscin within secondary lysosomes probably reflects phagocytosis by macrophages of degraded cell membranes bound to the lipid-soluble amiodarone. Once stored within the dermis this is clinically recognized by the typical blue-grey appearance which is likely to persist largely unchanged for many years. Photosensitivity may continue despite cessation of Am therapy because of the very considerable tissue retention of the drug. Furthermore the phototoxic reaction may be augmented by UVR excitation of locally stored granular Am and DA.

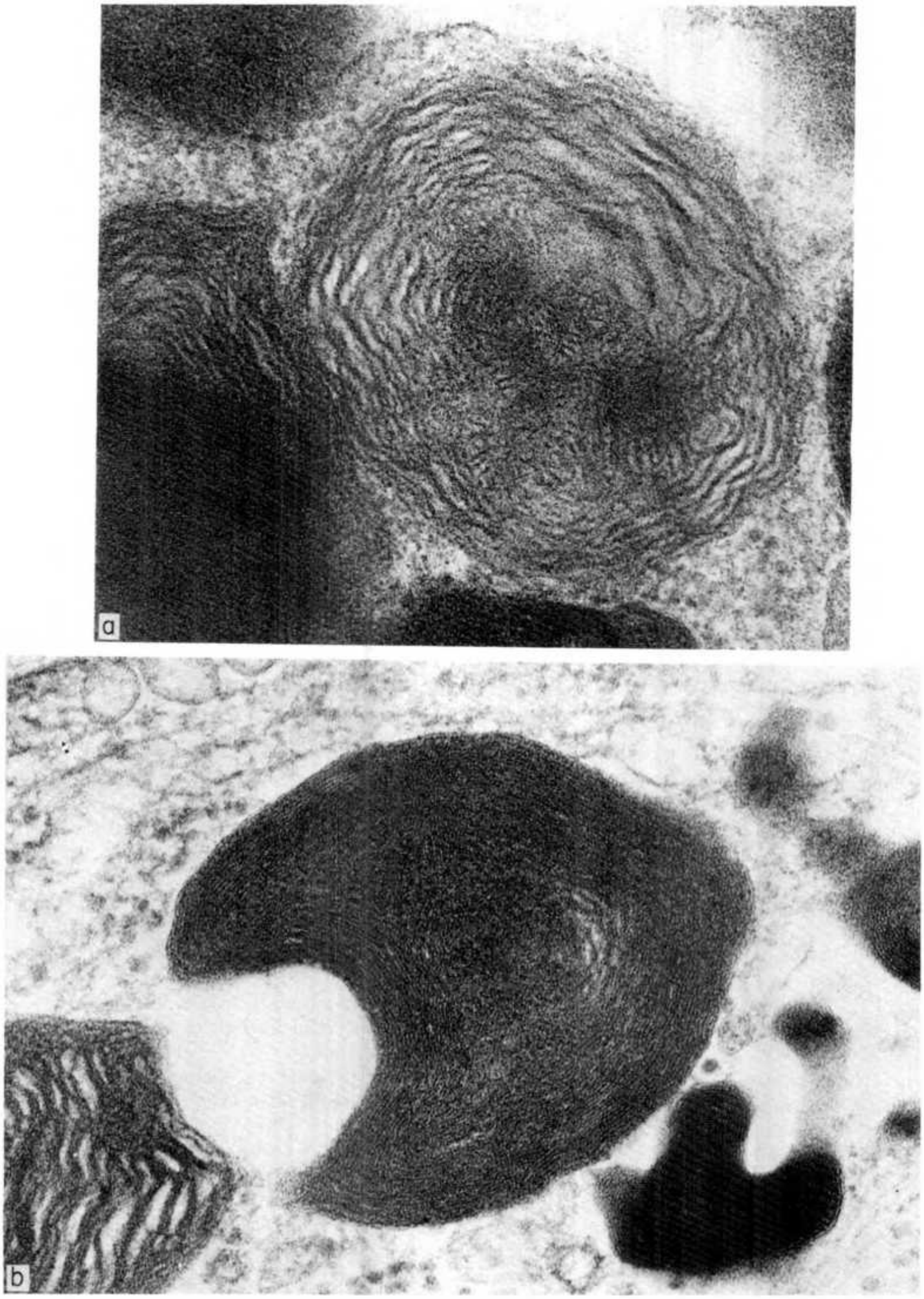

FIGURE 4. (a) Lamellated structures with central amorphous areas, TEM, (× 2880). (b) More compact lysosomes have a distinct fingerprint pattern with alternating electron-dense and electron-lucent zones and a regular periodicity of 12 Å, TEM (× 28,800).

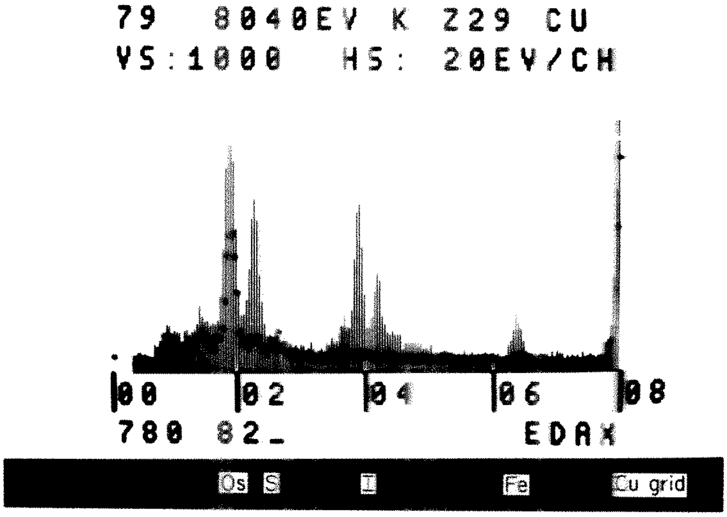

FIGURE 5. EDAX on light-exposed skin. Dark line shows test energy emission. Dotted black line shows background emission. Iodine (I) – 3·937, 4·220 KeV; sulphur (S) – 2·037 KeV; iron (Fe) – 6·398 KeV; osmium (Os) fixative – 1·914 KeV; copper (Cu) grid – 8·040 KeV.

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Pharmacology and Treatment

A study of hypoallergenic diets and oral sodium cromoglycate in the management of atopic eczema

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SUMMARY

Twenty-nine atopic children aged 3–12 years were given elimination diets for 3 weeks, individual groups of foods being then reintroduced over 6 weeks. Most children improved whilst dieting and relapsed on return to normal food intake. In all but 4 cases some food group which caused exacerbation of symptoms was identified, fruits and colourings proving more of a problem than milk and eggs.

Taking into account these suspected food allergens, tailored diets were designed for each child. Using these diets the group participated in a double-blind crossover trial of oral sodium cromoglycate (SCG) versus placebo. Twenty-two patients completed the trial. The results indicated that tailored diets were of value in the management of eczema but SCG did not produce a significant additional effect.

At follow-up 1 year later most children were adhering to their diet; thirteen were better and only three worse. Children who had initially continued using SCG had stopped it; no accompanying change in their eczema was noticed. A considerable reduction in the use of topical steroids was observed, in marked contrast to previous requirements.

Sequential measurements of total serum IgE (PRIST) showed variable changes apparently unrelated to recent SCG therapy. There was little agreement between skin prick tests and specific IgE (RAST) to corresponding foods. Correlation between RAST and food challenge was poor for positive results ($r = 0{\cdot}39$) but good for negative results ($r = 0{\cdot}99$).

In recent years interest has revived in the role of hypoallergenic diets in atopic eczema. Certainly there is increasing pressure from the lay public for information on the question of efficacy; unfortunately there are few adequately controlled trials on which to base an answer. Atherton and co-workers (1978) investigated the problem in a double-blind manner; however, their study was confined to the withdrawal of milk and eggs only. The improvement detected in their small

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group of patients was nevertheless encouraging. The detection of food allergy by means of elimination diets and subsequent food challenge is a laborious process. Reports on the usefulness of IgE levels or skin prick tests (Mackie *et al.*, 1979; Wraith *et al.*, 1979; Lessof *et al.*, 1980) have been conflicting and we felt further evaluation was necessary.

Hypoallergenic diets, even when tailored to individual requirements, are difficult to adhere to strictly and in the case of multiple allergies could well be nutritionally inadequate, especially in small children. Reports on the efficacy of oral sodium cromoglycate (SCG) in modifying symptoms of food allergy (Brostoff, Carini & Wraith, 1979; Wraith, Young & Lee, 1979) prompted us to undertake a double-blind study of this compound in conjunction with individually tailored diets.

The initial aims of the study were to determine whether or not an exclusion diet affects atopic eczema and whether particular food allergens could be identified by challenge tests or by specific IgE measurements. Following this, was it possible to design a hypoallergenic diet tailored for each patient, and would the use of such a diet with or without sodium cromoglycate be effective in the management of their eczema?

METHODS

The twenty-nine children participating in this study all had chronic atopic eczema necessitating regular attendance at an out-patient clinic. Their ages ranged from 3 to 12 years (mean = 7 years, 5 months) and there were nineteen boys and ten girls. Some of them had other symptoms of atopy such as asthma, rhinitis or urticaria. Written informed consent was obtained from their parents. The study period was 27 weeks and the protocol is shown in Table 1.

At the start of the lead-in period a full history was taken and the children were weighed and measured. Blood samples were taken for determination of total and specific IgE, total white cells and eosinophils. Similar samples were taken from an age-matched control group of six children, in hospital for minor surgery. All previous topical medication was stopped and the patients were given 1% hydrocortisone (labelled appropriately as 'The Cream' or 'The Ointment') and emulsifying ointment BP for use as required. This standard treatment was available throughout the study as was a colourless syrup given as a placebo in place of an antihistamine.

The patients were issued with a diary card for the daily subjective recording of pruritus, sleeplessness, the severity and area of eczema, and hydrocortisone and 'antihistamine' usage. All these were scored on a 4-point scale. The use of any other medication was also noted.

During weeks 3 and 4 of the study, all patients were placed on diet A. This was based on the diet devised at St Mary's Hospital, London (McEwen & Morgan, 1979) and excluded milk,

TABLE 1. Protocol of the study

2 weeks	Normal diet (pre-trial)
2 weeks	Exclusion diet A
1 week	Exclusion diet B
6 weeks	Challenge testing for major food allergens to determine tailored diet
2 weeks	Normal diet
6 weeks	Tailored diet plus cromoglycate or placebo
2 weeks	Normal diet
6 weeks	Tailored diet plus placebo or cromoglycate

eggs, cheese, fish, pork, bacon, offal, liver, shellfish, nuts, pips and fruits, juices and squashes, honey, sweets, chewing gum, gravy powder and yeast extracts.

For the next week they were subjected to the much harsher regime of diet B, allowing them to eat only sago flour, kosher margarine, lamb, carrots, celery, fresh green vegetables and rhubarb. Apart from water they could drink plain tea or home-made soda water.

There followed a 6-week challenge period when foods were reintroduced in groups as indicated in Table 2. The order of reintroduction was arbitrary and determined by the patient and the dietitian. The groups were reintroduced at intervals of 3 days. If an exacerbation of eczema occurred patients were asked to wait until it subsided before trying the next group. Throughout this stage of the study the clinicians were unaware of the order of reintroduction or of the patients' and parents' opinions of possible reactions to food groups. All this information was recorded by the dietitians.

From the results of the food challenges and specific IgE levels, an individual hypoallergenic diet was tailored for each child.

During the entire period of dietary restriction, calcium and vitamin supplements (in colourless form) were prescribed where needed.

Patients returned to a normal diet for 2 weeks prior to participating in a trial of the efficacy of SCG (Nalcrom®, Fisons) superimposed on their tailored diet. The trial was designed as a double-blind crossover, the patients being allocated in random fashion to treatment with SCG or a matched placebo which was identical in taste and form. During the first 3 weeks patients took one capsule q.d.s. before meals. They were instructed to dissolve the capsule in a little hot water and dilute to ½ cupful with cold water or a suitable drink. This was to be swilled round the mouth and swallowed. The dose was increased to two capsules for the next 3 weeks; each capsule contained 100 mg of drug. Patients then returned to a normal diet for a fortnight, after which they crossed over to the other treatment for a further 6 weeks. The children's heights and weights were determined again and fourteen of them volunteered another blood sample for measurement of total and specific IgE.

Throughout the study clinical assessment took place at defined intervals of 1, 2 or 3 weeks. The severity and area of eczema were marked on a homunculus and also scored on a 0–4 point scale; dryness and excoriation were also noted. If the eczema was infected enough to warrant antibiotic treatment, erythromycin by mouth was prescribed. This was a colourless preparation available from the hospital pharmacy. Local practitioners were advised of their patients'

TABLE 2 The eleven food groups reintroduced. Figures indicate the number of patients reacting (out of 22)

Nuts, pips, fruits, jams, fruit juices	14
Coloured drinks, sweets, jellies, instant puddings	9
Milk products, cheese, butter, ice cream	7
Egg and chicken	6
Yeast, soups, gravy	6
Pork, bacon, liver, sausages, beefburgers	6
Vegetables, peas, beans, melon, peanuts	5
Wheat, bread, flour, biscuits	4
Fish, tinned fish, fish fingers	4
Milk	2
Grains, rye, porridge, cornflakes	1

participation in the study and co-operated by not prescribing coloured drug preparations unless absolutely unavoidable. Patients were asked to bring their supplies of topical steroid and syrup to each clinic so that the amount they had used could be measured. They were also asked to bring any SCG or placebo capsules so that compliance could be assessed, and any other medicines in current use so that these could be recorded accurately.

Withdrawals

Of the original twenty-nine children, twenty-two completed the trial. A school skiing holiday accounted for two of the withdrawals; one mother considered it unacceptable for blood to be taken from her child; one patient refused to take the SCG; one failed to attend, and one was excluded from the analysis for non-compliance. The data from one other child were excluded from the final analysis because in the last week he was prescribed oral steroids for his asthma.

Follow-up

Those patients who completed the trial were followed up 1 year after it had started and on this occasion thirteen of them were skin prick tested and a sample of blood was taken for measurement of total and specific IgE. A further assessment was undertaken 1 year after the trial was completed, when eight children volunteered a third blood sample.

Measurement of serum IgE

Total serum IgE was measured by the paper radio-immunosorbent test (PRIST, Pharmacia), and specific IgE to nine common allergens by the radio-allergosorbent test (RAST, Pharmacia). The allergens used were *Anthroxanthum odoratum* (sweet vernal grass), cat epithelium, *Dermatophagoides pteronyssinus*, milk, egg white, codfish, peanut, brazil nut and wheat.

Prick tests

Patients were asked to refrain from taking any antihistamine for 3 days prior to the test. Tests were performed on the volar surface of the lower arm with the following antigen solutions (Bencard): grass, *Aspergillus fumigatus*, *Dermatophagoides pteronyssinus*, cat epithelium, dog epithelium, feathers, egg white, milk, cheese, codfish, mixed nuts, peas, wheat, chocolate and orange.

Analysis of results

Statistical analysis of the results was performed by the statistics department of Fisons, as detailed in the results section.

RESULTS

White blood cell count

The mean white cell count for the group was $10{\cdot}5 \times 10^{9}$/l (range 6·7–17·3). Most children showed a mild eosinophilia, the mean value being 9% of the total white cell count with a range of 1–21%.

Height and weight

All the children grew during this time. Only three of them failed to gain weight, two remaining the same and one falling from 18·7 kg to 18·0 kg over the 27 weeks.

Effect of exclusion diets on severity and area of eczema

As can be seen in Table 3, thirteen children improved on diet A; changing to diet B gave further improvement in nine of these, though two became worse. Of the four children whose eczema showed no change on diet A, two improved on diet B. Five children became worse on diet A but all of them improved markedly on diet B. Thus over the entire period of dietary restriction eighteen children improved, three showed no change and one became worse. This clinical assessment, based on single observations made before and after each dietary period, agreed well with the parents' assessment based on daily diary card scores. Fifteen patients reported less itching whilst they were on the diets. Moreover, changes in personality were reported in several cases, school teachers commenting on improved concentration, behaviour and a 'happier disposition'.

Food challenge

Foods were reintroduced into the diet a group at a time. These groups are outlined in Table 2 which also gives the number of children reacting to any group by an exacerbation of their eczema. The main culprit was the nuts, fruits and jams group followed by that containing artificially coloured and flavoured foods. Eggs and milk were less of a problem. Overall eighteen of the twenty-two children showed a definite reaction to one or more food groups. In addition the occasional urticarial reaction was observed. No further attempt was made at this stage to identify individual foods more positively. When they all returned to a normal diet twenty relapsed including two in whom a definite food allergen had not been detected.

Tailored diet and SCG or placebo

The overall results of the trial are shown in Table 4. It can be seen that the severity and area of eczema decreased while the children were on the exclusion diets. This reduction was significant during week 5 (Friedman non-parametric analysis of variance; $P < 0{\cdot}01$). On return to a normal diet there was an exacerbation of eczema in the group as a whole, the scores being similar to those of the pre-trial period.

Adherence to a tailored diet was also of benefit in reducing the severity of eczema significantly ($P < 0{\cdot}01$), though there was little change in the area involved. The addition of SCG did not enhance the beneficial effects of the tailored diet as can be seen by comparing the scores with those from the placebo. Nevertheless fourteen patients wished to continue with SCG treatment.

TABLE 3. The effects of exclusion diets on eczema. The numbers in parentheses represent the parents' assessment

Normal diet	Diet A	Diet B	Overall
22	13 Improved	9 Further improvement 2 Maintained improvement 2 Worse*	18 (15) Improved
	4 No change	2 Improved 2 No change	3 (4) No change
	5 Worse	5 Improved	1 (3) Worse

* But one still better than on normal diet.

TABLE 4. The effect of exclusion diets and SCG on eczema. (Sodium cromoglycate 100 mg q.d.s. for 3 weeks, then 200 mg q.d.s. for 3 weeks.)

Weeks of trial	Treatment	Mean eczema score (maximum = 4) Severity	Area
1,2	Lead-in period, normal diet	2·09	1·98
3,4	Exclusion diet A	1·68	1·77
5	Exclusion diet B	1·20*	1·41
12,13	'Normal' diet	1·91	2·11
14–19	Tailored diet + placebo	1·40*	1·88
22–27	Tailored diet + cromoglycate	1·46*	1·82

* Significant reduction in severity ($P < 0{\cdot}01$).

Steroid usage

In all but one patient the only steroid needed during the study was 1% topical hydrocortisone, in marked contrast to previous requirements for more potent medication. The amounts used each week hardly varied and bore no relationship to the severity of the eczema. This reduced use of stronger steroids carried over into the follow-up period, only two of the patients needing them during that year, and both had forsaken their diet.

Follow-up

By 6 months after the trial the fourteen patients who had persevered with SCG had all stopped using it, with no corresponding change in their eczema. Of the complete group two children had suffered acute exacerbations and one child a slow deterioration, but the others were continuing to improve. Nineteen of the original (22) children were assessed after a further 6 months. Eleven of them were still adhering to their diet and two had never been restricted. The results are shown in Table 5. Thirteen were better than at the end of the trial, three were unchanged and three slightly worse. Overall, seventeen of the nineteen were better than at the beginning of the study some 18 months earlier.

TABLE 5. Assessment of the severity of eczema in nineteen patients made at follow-up a year later, compared with the assessment made at the end of the trial. Compared with the beginning of the trial 18 months earlier, seventeen patients had improved and two showed no change

Assessment of eczema	Adherence to tailored diet	Rejection of tailored diet	Normal diet because no identification of food allergens	Totals
Improved	9	3	1	13
No change	1	1	1	3
Worse	1	2	—	3
Totals	11	6	2	19

Total IgE

The initial total serum IgE levels ranged from 24·0 to 44,000 units/ml with a mean of 4860 (Fig. 1), significantly greater than those of the age-matched controls whose values ranged from 7·0 to 290 units/ml, with a mean of 65·5. At the end of the trial the IgE was measured again in fourteen patients; changes varied from child to child and were not apparently related to whether they had spent the preceding 6 weeks on SCG or placebo (Fig. 2). Of the seven children who had been receiving SCG, the IgE levels were raised in four cases, of whom three were asthmatic and had all experienced a recent attack. The remaining three showed little change; one of them was asthmatic but had not been affected recently. The pattern was absolutely identical in the seven children who had been in the placebo group. Subsequent measurements in some of these children 5 months later showed the total serum IgE levels to be comparable to those determined 1 year earlier.

Specific IgE

Specific IgE (RAST) was measured to six food and three inhalant allergens. Those patients with high total IgE not surprisingly had multiple positive RAST responses (Fig. 1); they also had the worst eczema. A detailed analysis of the RAST results is given in Fig.3. As far as the food allergens are concerned, note the preponderance of high scores to nuts compared with those to milk and egg. Only one control subject had a positive score to a food. The vast majority of the children (twenty-two out of twenty-nine patients and one of the controls) were strongly positive to house dust mite. Table 6 enumerates the relationship between RAST responses and food challenge. An apparently poor correlation was obtained between positive RAST and positive food challenge ($r = 0{\cdot}39$) but this is an incomplete and thus biased summary of these results because some patients with a positive RAST knew they were allergic to the particular food and

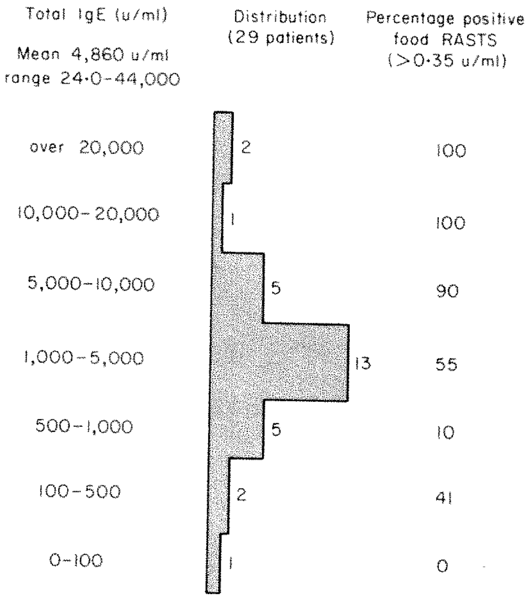

FIGURE 1. Total serum IgE (PRIST) levels in twenty-nine children with atopic eczema. The incidence of elevated levels of specific IgE (RAST) to 6 foods is also given. The mean value for a group of age-matched controls was 65·5 u/ml, range 7·2–290.

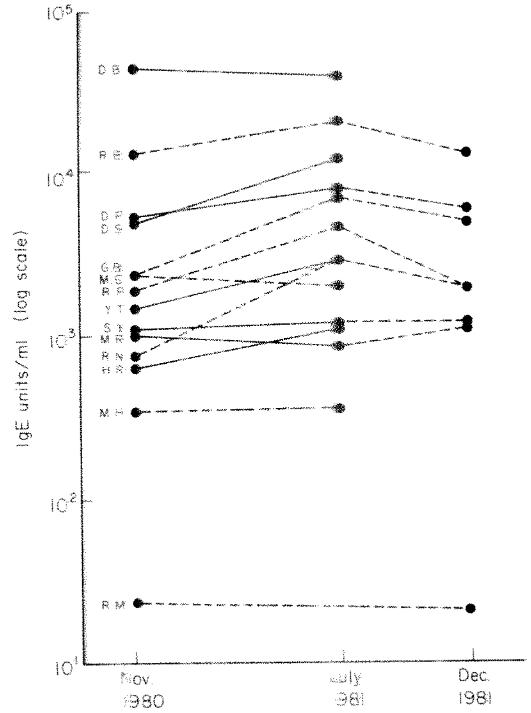

FIGURE 2. Serum IgE levels measured in volunteers at the beginning and end of the clinical trial and at follow-up 1 year later (December 1981). Solid lines indicate those who had been on sodium cromoglycate in the last 6 weeks, hatched lines those on placebo.

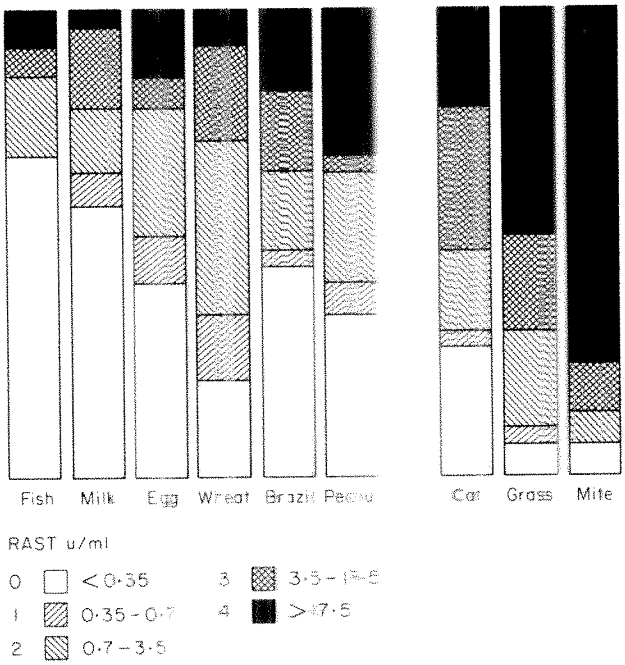

FIGURE 3. Details of the distribution of specific serum IgE levels (measured by RAST) to six food and three inhalant allergens in twenty-nine patients. (All RAST scores for five out of six age-matched controls were zero, the sixth scored 1 to egg and 4 to house dust mite.)

TABLE 6. Predictive value of RAST versus food challenge

	Egg	Milk	Wheat	Fish	Peanut	Brazil nut	Totals
Positive RAST	14	11	14	8	10	4	61
Positive challenge	6	2	4	4	6	3	25
Negative challenge	8	9	10	4	4	1	36
Negative RAST	11	14	4	18	7	11	65
Negative challenge	10	12	4	16	7	11	60
Positive challenge	1	2	0	2	0	0	5

Correlation coefficients: Positive against positive $r = 0{\cdot}39$; Negative against negative $r = 0{\cdot}99$.

understandably failed to challenge with it. The correlation between negative RAST and negative food challenge was good ($r = 0{\cdot}99$).

Skin prick tests

Skin prick tests were performed in thirteen patients on the same day a blood sample was taken for IgE determination. There was little agreement between the two sets of data, the correlation coefficient for positive results being 0·49 for foods and 0·71 for inhalants. There was even less agreement between prick tests and food challenge results.

DISCUSSION

This study supports the view that food allergy can play an exacerbating role in childhood atopic eczema. It is perfectly possible to identify broad groups of suspect foods in the context of a busy out-patient clinic in the manner we have described. Some children reacted within hours of ingesting a particular allergen but some did not react for 2 or 3 days. Thus, with hindsight, reintroduction of foods at intervals of less than a week is not recommended.

Although there was a significant improvement in the patients' eczema whilst on strict elimination diets, these are obviously quite unsuitable for long-term use. However, by tailoring a diet for each individual child the improvement was maintained. When SCG was superimposed on this diet in the double-blind crossover trial against placebo no additional benefit was seen. This trial design was chosen because the relatively crude identification of potential food allergens undoubtedly left some undetected. It was thought that oral SCG might protect against these. This lack of effect of sodium cromoglycate was also noted by Atherton, Soothill & Elvidge (1982), though others (Molkhou & Waguet, 1979; Cavagni, 1981) have found it helpful in some cases. We are in the process of evaluating the effects of SCG in adult atopics on normal diets.

Atherton's earlier study (1978) was confined to milk and eggs; these may well be of prime importance in infants under 2 years but we did not investigate this age group. The present results place artificial colourings, preservatives and citrus fruits top of the list of offending foods. An association between these and urticaria is well documented (August, 1979) and in a few of our patients an early urticarial reaction was seen before the eczematous response.

It was our experience that skin prick tests were not helpful in the search for food allergens and

RAST measurements of specific IgE levels failed to provide an easy laboratory answer. Clinical challenge remains the only reliable test.

An interesting observation was the high incidence of a strongly positive RAST response to house dust mite, coupled in most instances with a positive prick test. Two of the patients responded to superficial contact with a test solution of house dust mite. Mitchell *et al.* (1982) claimed that they could produce an eczematous lesion in response to a modified patch test with the P_1 antigen of house dust mite faeces. We believe that contact with, and indeed, ingestion or inhalation of house dust mite in relation to eczema needs attention (Graham *et al.*, 1982).

It has been reported by Mackie (1981) that IgE levels fell in some patients after receiving treatment with oral SCG. In a few instances this could be related to an improvement in eczema. Our own results in fourteen patients do not agree with this. If anything, levels tended to rise in both the treated and placebo groups. However, these measurements were made in July and the increase in total IgE levels occurred predominantly in asthmatic children who were either suffering wheezing episodes at the time, or had just recovered from an acute attack. Neither of the asthmatic subjects with unchanged IgE had suffered a recent bout of asthma, and only two non-asthmatic subjects showed a rise in total IgE levels. Measurements taken the following winter in seven of these subjects showed that the higher levels obtained in the summer had fallen and were comparable to those taken previously at the same time of year. None of these seven had taken SCG during the preceding 8 weeks. Thus, the present evidence suggests that this was a seasonal change. None of the increases or decreases in serum IgE apparently corresponded with exacerbation or remission of eczema. However, very recently, a large-scale study in atopic eczema patients has indicated that a subset of such a population does show a fall in IgE levels, together with a concurrent improvement in eczema when treated with oral SCG (Edwards, personal communication). More sequential measurements of serum IgE levels are in progress in a larger number of patients.

Elimination and challenge diets are time consuming and demand a high standard of co-operation from parents and patients. Whilst we recognize that the natural history of eczema in children is frequently one of improvement, nevertheless our results sustained over 18 months lead us to believe that such diets are worth undertaking in the more severe cases. If the necessity for strong topical steroids can be reduced by this means it is surely an advantage.

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Dermatological aspects of the use of Cyclosporin A for prophylaxis of graft-versus-host disease

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SUMMARY

Oral Cyclosporin A (CyA) was given for the prophylaxis of graft-versus-host disease (GVHD) to sixty-seven patients receiving a bone marrow transplant, and was found to be extremely effective in reducing the severity of acute GVHD. Side-effects of CyA included hypertrichosis, gum hypertrophy, and a rare but serious 'capillary leak' syndrome.

Until 1980, methotrexate was the principal agent used for the prophylaxis of graft-versus-host disease (GVHD). At Westminster Hospital, between 1980 and 1982, Cyclosporin A (CyA) was given to prevent GVHD in sixty-seven patients receiving a bone marrow transplant. During this 2-year period there was a marked improvement in survival figures. This paper presents our clinical observations on the dermatological aspects of CyA therapy.

The primary target organs of GVHD are the skin, the gastro-intestinal tract and the liver. The cutaneous manifestations of GVHD have been reviewed recently (Roujeau, Revuz & Touraine, 1980; Harper *et al.*, 1983). Once established, GVHD is usually treated with high doses of intravenous methylprednisolone, the dosage being titrated against the clinical response.

PATIENTS

Of the sixty-seven patients, forty-six had acute leukaemia, eight had aplastic leukaemia and thirteen had inborn errors of metabolism. Fifty-three received transplants from HLA identical MLC-matched family donors, nine from haplotype identical mismatched family donors and five from fully matched but unrelated donors. Forty-five patients are alive. The various dosage schedules of CyA are shown in Table 1.

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TABLE 1. Comparison of Cyclosporin A schedules for prophylaxis of graft-versus-host disease (Barrett *et al.*, 1982a)

Schedule	Dose of CyA	No. of patients (series of 36)
I	12·5 mg/kg (days −1 to +100)	10
II	25 mg/kg (days −4 to +100)	6
III	12·5 mg/kg (days −4 to +100)	13
IV	12.5 mg/kg (days −10 to +100)	7

RESULTS

The overall incidence of GVHD for all the bone marrow transplants performed at Westminster Hospital, from 1970 to 1982 inclusive (total 114), was 76%. Of the sixty-seven patients who received CyA, fifty-one (76%) developed acute GVHD and nineteen (28%) developed chronic GVHD (Table 2). The incidence of acute GVHD, therefore, was unaltered but its severity was significantly reduced.

TABLE 2. Cyclosporin A prophylaxis of graft-versus-host disease at Westminster Hospital (1980–1982)

	Graft-versus-host disease					
No. of patients	None No. (%)	Skin No. (%)	Gut No. (%)	Liver No. (%)	Chronic skin No. (%)	Chronic liver No. (%)
67	16 (24)	51 (76)	30 (45)	12 (18)	19 (28)	1 (1)

TABLE 3. Comparison of methotrexate and Cyclosporin A prophylaxis of graft-versus-host disease in acute leukaemia (Age- and sex-related groups; sex-matched donor–recipient pairs)

		Graft-versus-host disease					
Graft-versus-host disease prophylaxis	No.	None No. (%)	Skin No. (%)	Gut No. (%)	Liver No. (%)	Chronic skin No. (%)	Chronic liver No. (%)
Methotrexate	7	0 (0)	7 (100)	5 (71)	5 (71)	0 (0)	0 (0)
Cyclosporin A	7	2 (29)	5 (71)	3 (43)	0 (0)	2 (29)	0 (0)

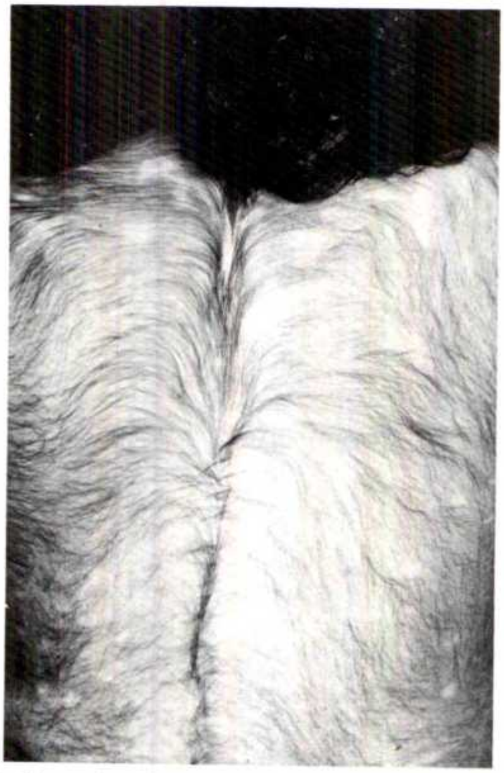

FIGURE 1. Hypertrichosis of the neck and upper back due to Cyclosporin A. The hypopigmented areas are due to chronic graft-versus-host disease.

Seven patients with acute leukaemia, who received prophylactic methotrexate prior to May 1980 were compared with seven patients receiving CyA. The mean ages, sex of recipient and disease type were comparable in the two groups (Table 3). The decreased severity of acute GVHD was reflected by the reduced duration of treatment with methylprednisolone. The methotrexate group required methylprednisolone for a mean of 17 days (range 5–32 days) compared with a mean of 3 days (range 0–7 days) for the CyA group ($P < 0{\cdot}01$).

A common side-effect of CyA was hypertrichosis, affecting 80% of the patients with a variable degree of increased hair growth on the face and body. The sites of predeliction are the face, particularly the eyebrows, the upper part of the back maximal along the line of the spine (Fig. 1) and the lateral aspect of the upper arms. This was usually noted after about 8 weeks post-graft and was slowly reversible after the drug was stopped.

A severe 'capillary leak' syndrome was witnessed in five patients on CyA, three of whom died. This was associated with marked fluid retention and periorbital oedema, and in some, gastro-intestinal bleeding and pneumonitis. It was preceded by a rapid rise in urea, massive urinary sodium loss followed by hypertension and convulsions (Joss *et al.*, 1982). They also had a low central venous pressure.

DISCUSSION

Cyclosporin A is a potent immunosuppressive agent that is now widely used in organ transplantation, as well as in the prophylaxis of graft-versus-host disease (GVHD) following allogeneic bone marrow transplantation.

Cyclosporin is a metabolite isolated from the culture broths of the fungal species *Tolypocladium inflatum Gams*, previously known as *Trichoderma polysporum (Link ex Pers.) Rifai*, and is a cyclical polypeptide of eleven amino acids. Cyclosporin A was discovered in 1972, in the laboratories of Sandoz (Switzerland) in a search for biologically produced anti-fungal agents. Although the compound proved to have only mild anti-fungal activity, it was found to have a number of immunological properties (Borel *et al.*, 1977).

CyA affects predominantly T-lymphocyte dependent immune responses. The available data from *in vitro* and *in vivo* investigations suggest that CyA has a sparing effect on the activation and establishment of suppressor cell regulatory mechanisms while inhibiting cytotoxic T-lymphocyte generation in response to transplantation antigens (Hess, Tutschka & Santos, 1982). This imbalance is in favour of transplantation tolerance. Unlike most other immunosuppressants, CyA is relatively non-toxic to the bone marrow. Calne and White (1977) in Cambridge first demonstrated the ability of CyA to prolong organ graft survival in man.

CyA and GVHD

CyA has been shown to be active against GVHD, in particular in prophylaxis (Powles *et al.*, 1978, 1980; Barrett *et al.*, 1982b). CyA may prevent the expansion of donor T-lymphocyte clones in response to the new antigens of the host tissue, which are responsible for acute GVHD (Palaciso & Molle, 1981). CyA has proved to be extremely effective in reducing the severity but not the incidence of acute GVHD. There is a better survival rate and decreased severity of GVHD in patients receiving prophylactic CyA compared with methotrexate (Kendra *et al.*, 1981). Interestingly, the incidence of chronic GVHD of the skin is slightly higher in the CyA group, which probably reflects the better survival figures and improved clinical recognition, in particular oral lichen planus and the subtle rash of lichen planopilaris. Chronic GVHD shows less response to CyA, and a combination of prednisolone and azathioprine is the present treatment of choice. CyA is, therefore, used to modify acute GVHD (treatment is usually stopped 100 days post-graft at Westminster Hospital).

Although oral CyA seems effective in reducing the severity of acute GVHD, prevention is limited by toxicity of the drug and variable absorption. It is not possible to define an effective therapeutic level of CyA, although most data suggest that sustained plasma levels of over 200 ng/ml are required. Barrett *et al.* (1982a) have reported their failure to modify the incidence and severity of acute GVHD using different dose schedules of CyA (Table 1) in an attempt to obtain high CyA levels at the time of the transplant. Plasma CyA levels do not appear to be more useful in individual patients in predicting toxicity, than the regular measurement of blood urea, creatinine, blood pressure and body weight. Better recognition of the early side-effects are important as these are usually reversible with dose reduction.

Skin changes caused by CyA

Hypertrichosis (sometimes referred to as hirsutism in the non-dermatological literature). This common side-effect is not usually a significant problem. Stimulation of hair growth on nude mice by CyA has been reported by Pendry and Alexander (1982). Examination showed no significant abnormality in the gross architecture of the hair shaft. Under plane-polarised transmitted light the hairs were birefringent, i.e. normal. The only unusual finding was that the hairs tended to be round at the tip, unlike the distal end of normal hairs which usually appear as a blunt point. This may reflect changes in the rate at which the hair moves through the pro-anagen phase of the hair cycle. The hairs were easily plucked, reflecting the status of the anagen root. Many of the hair follicles had fine, rather abortive roots.

Gum hypertrophy. This is a well-described side-effect of CyA seen frequently in renal transplant patients (Calne, Rolles & White, 1981), but encountered in only one of our patients.

Capillary leak syndrome. This serious disorder has been attributed to CyA toxicity (Barrett *et al.*, 1983). A generalized increase in capillary permeability causes multiple organ damage (lungs, skin, gut etc.). We have seen this in five patients (7·5%), three of whom died.

Other side-effects. Some degree of renal impairment, as shown by a rise in blood urea and creatinine, occurred in 75% of patients overall. This effect was reversible in all but four patients who developed oliguric renal failure. Two of these patients died with multiple problems. In addition and particularly in children, hypertension was frequently observed. In a study of twenty-nine of our patients sixteen (55%) had a diastolic blood pressure of greater than 100 mm Hg at some stage after transplantation (Joss *et al.*, 1982). Seven children had convulsions in association with hypertension and fluid retention. Tremor is seen only in a few with severe toxicity.

Rolles and Calne (1980) reported two cases of benign breast lumps (fibroadenomata) in patients on CyA therapy after receiving a kidney graft.

Fifteen cases of lymphoma have been reported out of 1800 patients throughout the world who have received CyA (Iglehart, 1983); eight in patients with renal transplants, six in patients with cardiac transplants and one in a patient with a bone marrow transplant. The incidence of lymphoma appears to be no greater than that seen with other immunosuppressive agents. There is evidence that this could be associated with the reactivation of Epstein-Barr virus (Crawford *et al.*, 1980). None of our patients has yet developed a lymphoma.

This review of the dermatological aspects of CyA therapy would not be complete without mention of other dermatoses in which it has been used.

CyA and Sézary syndrome

CyA has been used in the treatment of Sézary syndrome. A patient, treated at Westminster Hospital, improved initially both clinically and with a reduction in the absolute Sézary count, but after 4 weeks he escaped from treatment with a sudden rise in the number of circulating Sézary cells. The CyA was discontinued and he was started on bleomycin; however, within 2 months he developed 'bleomycin lung' and died. Catterall *et al.* (1983) have reported a 28-year-old man with Sézary syndrome, who developed an aggressive T-cell lymphoma 8 months after starting CyA, suggesting that the drug may accelerate or adversely alter the natural history of the disease.

CyA and psoriasis

Mueller and Herman (1979) used CyA to treat four patients with severe psoriasis and noted that the psoriatic plaques disappeared only 5 days after starting treatment, but gradually reappeared after cessation of treatment, only to resolve again when the treatment was restarted. There have been no other reports of this observation.

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The mode of action of ethyl lactate as a treatment for acne

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SUMMARY

We have shown that an alcoholic lotion containing ethyl lactate when applied topically to rat skin under occlusion became localized in the follicles and sebaceous glands. When applied to human facial skin the ethyl lactate was hydrolysed to ethanol and lactic acid, and thereby lowered the skin pH. Under such conditions the growth of recoverable skin bacteria, in particular the anaerobe *Propionibacterium acnes*, was inhibited, and the hydrolysis of sebum to free fatty acids by lipase derived from the bacteria was greatly impaired. These effects of ethyl lactate would account for its observed clinical efficacy in acne vulgaris.

The mode of action of ethyl lactate in the treatment of acne was originally postulated by Swanbeck (1972) to involve free fatty acids, which at that time were held to be important in producing the comedonal and inflammatory phases of the disease (Kligman & Katz, 1968). Swanbeck proposed that ethyl lactate, delivered topically from a water-free system, would penetrate sebaceous follicle ducts, wherein much would be hydrolysed to ethanol and lactic acid, thereby lowering follicular pH, and as a consequence the bacterial lipolysis of sebum triglycerides to free fatty acids would be reduced. In the absence of evidence of clinical efficacy ethyl lactate was considered to be novel in concept, but not adequately tested (Cunliffe & Cotterill, 1975).

More recently, clinical evidence of the efficacy of a hydro-alcoholic lotion of ethyl lactate in acne vulgaris has been published by Grosshands, Fourtanier & Guidbaud (1978). This finding has prompted us to investigate more fully the proposed mode of action of ethyl lactate in the light of current knowledge of acne pathogenesis (Scott, Cunliffe & Gowland, 1979). A preliminary description of these findings has been given elsewhere (Prottey *et al.*, 1983).

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METHODS

Penetration of ethyl [^{14}C]lactate in rats

Ethyl [^{14}C]lactate was synthesized from sodium L-[U-^{14}C]lactate (Radiochemical Centre, Amersham) and 'super-dry' ethanol, and was shown to be more than 99% pure by thin-layer and radio gas–liquid chromatography.

The hair was clipped from the backs of twenty-four female Colworth Wistar rats (weighing 130–150 g), and 24 h later 100 μl of a solution of the 10% ethyl lactate lotion (see below) that had been spiked with 21×10^6 d.p.m. ethyl [^{14}C]lactate (i.e. 1 g ethyl lactate = 2150 d.p.m. of ^{14}C) was applied to 6 cm^2 dorsal skin from a microsyringe.

Groups of four rats were killed immediately and at 0·5, 1, 2, 6 and 24 h after application. Those rats in the 0·5, 1 and 2 h treatment groups were fitted with restraining collars and those in the 6 and 24 h groups with polythene-lined protective patches, devices to prevent the animals licking the treated skin.

For autoradiography the skin was excised immediately and snap frozen between flat blocks of solid CO$_2$. Fresh frozen sections (8 μm) were cut in a cryostat at −20°C and mounted on emulsion-coated histological slides. Exposure to the emulsion was for 14 days at −20°C, at which time the emulsion was developed and the skin section fixed and stained with haematoxylin and eosin.

Autoradiograms were photographed using dark field illumination, so that the developed silver grains indicating the localization of radioactivity appeared as white spots against the stained section of skin.

Human subjects and treatments

All measurements were performed on male and female laboratory personnel with normal skin and in the age range 18–40 years. The number participating is specified below for each experiment: no attempts were made to discriminate between male and female subjects.

The ethyl lactate lotion comprised 10% ethyl lactate L(−) dissolved in 44% ethyl alcohol and 2% lactic acid L(+), and neutralized to pH 5·3 with triethanolamine. The base vehicle that was used as a control contained no ethyl lactate, but was similarly adjusted to pH 5·3 with 2% lactic acid and triethanolamine. We also used 44% ethanol in water as a further control, with no buffering or pH adjustment.

Treatment consisted of applications by means of cotton wool moistened with the respective lotion, and gently wiped over the forehead and cheeks; this was repeated. Subjects applied the lotions twice daily, in the morning and before retiring. They were instructed to wash their faces with soap and water before application of the lotion. Treatment was continued daily for 2 weeks, and measurements/samples taken beforehand, after 1 week, after 2 weeks, and after a further week following cessation of application. On the day that measurements/samples were taken, this would be before the first daily face washing and lotion application (i.e. overnight from the previous application). The three lotions were examined sequentially over a 3-month period, and treatments were randomized to obviate any seasonal variation in the measurements.

Skin pH measurement

Skin pH of the mid-forehead region was determined using an Activion CTF75 Combination flat membrane pH electrode and a Radiometer PHM71 meter. The electrode was stored in 0·1% saline, adjusted to pH 5·0 with traces of dilute HCl. When placed upon the skin sufficient saline was carried over to ensure conduction between the probe and the reference. Stable readings were generally obtained within 15 s.

Microbiology

Samples of skin micro-organisms were obtained using a modification of the scrubbing technique of Williamson & Kligman (1965), using sterile phosphate buffer (0·1 M, pH 7·9, 0·1% Triton-X-100) in chambers of area 10·8 cm^2 on the mid-forehead for 1 min.

Thioglycollate and blood agar plates were inoculated with suitable dilutions of forehead scrubbings using the spread plate technique of Postgate (1969). Blood agar plates were incubated aerobically at 37°C for 48 h. The thioglycollate plates were incubated anaerobically at 37°C for 7 days. Subcounts of the various colony morphologies were then taken (Cunliffe & Cotterill, 1975).

Of those colonies that were Gram-positive diptheroidal rods, samples were inoculated into thioglycollate broth and streaked on to milk agar plates to test for casein hydrolysis. Aliquots of the incubated broth were inoculated into the sucrose fermentation test, the trehalose fermentation test and the gelatin liquefaction test (Cowan & Steel, 1974). From these tests *Propionibacterium acnes* and *Propionibacterium granulosum* were identified.

Skin surface lipase/esterase activity

Samples for the enzymic assay of lipase/esterase activity were taken from the skin surface scrubbings, cloudiness being removed by passage through Millipore membranes (0·45) before assaying. A 1-ml portion of the filtrate was added to 0·4 ml phosphate buffer (0·5 M, pH 7·5) and $6{\cdot}5 \times 10^{-4}$ M 4-methyl umbelliferyl butyrate in water. After incubation at 37°C for 1 h liberated 4-methyl umbelliferone was determined using a Locarte® fluorometer ($\lambda_{ex} = 360$ nm; $\lambda_{em} = 460$ nm), by comparison with standard solutions.

Sebum free fatty acid: triglyceride ratios

Sebum samples were taken from the forehead by means of frosted glass slides (Schaeffer, 1973). After extraction and purification of the lipids of the sebum (Folch, Lees & Sloane-Stanley, 1957) thin-layer chromatographic separation into constituent lipid types was performed using silica gel G plates developed at room temperature in diethyl ether:hexane:acetic acid (30:70:1, by vol) halfway up the plate, followed by development in diethyl ether:hexane: acetic acid (10:90:1, by vol). Lipids so separated were charred with 50% sulphuric acid at 180°C for 15 min, and the lipids were quantified by photodensitometry using a Zeiss Spectrophotometer.

RESULTS

Penetration of ethyl lactate

From autoradiograms of treated rat skin visual assessment of silver grain density was performed: data are presented in the Table 1. The follicles and sebaceous glands contained relatively high proportions of radioactivity from the topically applied ethyl [^{14}C]lactate. At times up to 2 h the epidermis showed sparse labelling, and this is a reflection of the volatile nature of the ethyl lactate and the open application. At 6 and 24 h the epidermal labelling was increased, as was that in the follicles and subaceous glands: this reflected the use of occlusive patches on the rats at these longer times (see methods). These data demonstrate that shortly after application of ethyl [^{14}C]lactate to the skin, ^{14}C had accumulated in the hair follicles and sebaceous glands and persisted for several hours.

TABLE 1. Distribution of silver grain density in autoradiograms of rat skin treated with ethyl [^{14}C]lactate

	Time (h) after application				
Location	0·5	1	2	6	24
Epidermis	0–1	0–1	0–1	2–3	2–3
Dermis	0–1	0–1	0–1	1–2	2
Hair follicles	1–2	2	1–2	2	2
Sebaceous glands	1–3	2–3	2–3	3–4	3–4

0 = nil; 1 = low; 2 = moderate; 3 = moderately heavy; 4 = heavy.

Fig. 1 illustrates representative tissue samples at 24 h after application.

Reduction of pH of human skin surface

Fig. 2 illustrates an experiment in which skin pH was measured at various times with the sequential re-application of ethyl lactate lotion to the foreheads of volunteers. This graph shows the pretreatment equilibrium pH of the forehead surface to be of the order of 5·5, which is a well-documented value (Blank, 1939). Sequential application of 40% aqueous ethanol alone caused little or no change in skin surface pH, whereas application of the ethyl lactate lotion

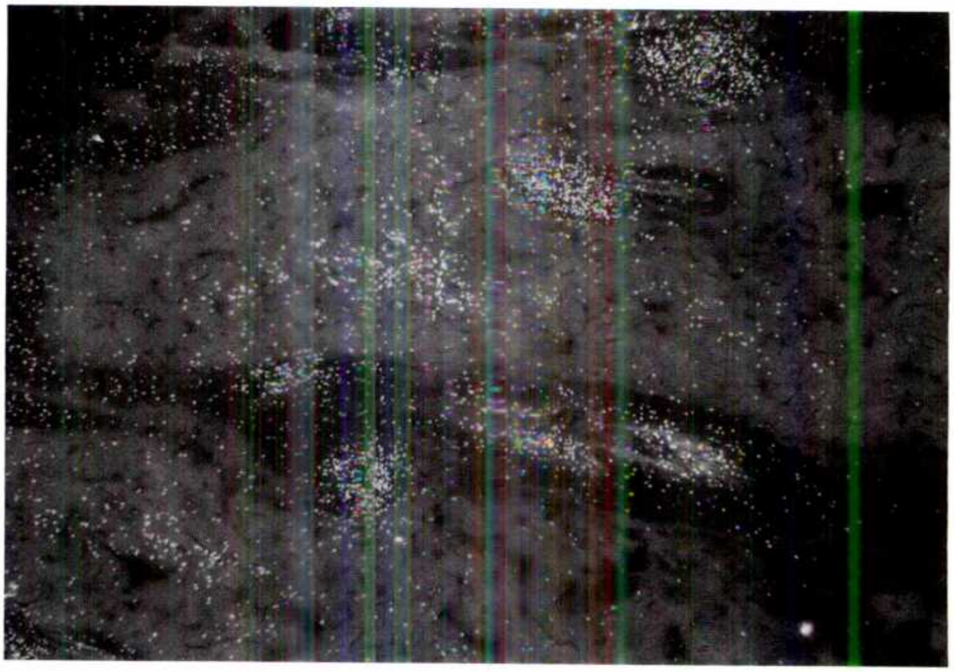

FIGURE 1. Rat skin 24 h after application of ethyl [^{14}C]lactate. The sebaceous glands show the presence of radioactivity from the applied material as white dots. (Mag. × 200.)

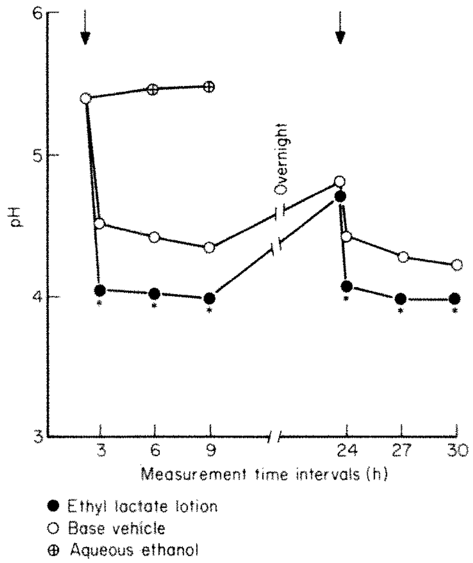

FIGURE 2. Effect of ethyl lactate lotion upon human forehead skin pH. The values plotted are the means of observations in twelve subjects. Application to the skin is marked by arrows. Asterisks indicate statistically significant lower values, relative to the base vehicle control ($P<0{\cdot}05$).

significantly lowered the skin surface pH to a value of about 4·0 ($P<0{\cdot}01$). Subsequent applications of the ethyl lactate lotion at 3-hourly intervals failed to lower the surface pH below 4·0, thereby indicating that even under excessive usage skin surface pH would not be lowered unduly. During overnight recovery the skin surface pH was observed to rise towards the equilibrium value: repeated applications on the following day re-introduced the pH lowering effect. By comparison, the effect of the base vehicle (i.e. containing no ethyl lactate, but adjusted to pH 5·3, with lactic acid) is shown. This too had some capability for lowering skin surface pH, but was by no means as effective as the ethyl lactate-containing lotion.

In order to establish that a reservoir of low pH material was present in the skin, successive sellotape strippings were taken from the treated skin surface of the forearm. Each tape strip removed one to two layers of the stratum corneum (there being an average of twenty to thirty such layers normally), and Fig. 3 shows that the ethyl lactate lotion produced a sustained lower pH of the stratum corneum compared with skin treated with the base vehicle. This supports the concept that ethyl lactate delivers a low environment to the skin and so, by inference, to the sebaceous follicles.

Effect of ethyl lactate upon bacteria of human skin

Skin surface scrubbings were taken from the foreheads of volunteers at various times during the application of the ethyl lactate lotion, and typical data are shown in Fig. 4 for the recoverable aerobic bacteria and Fig. 5 for the anaerobic bacteria. The reduction in recoverable aerobes during a 2-week period of application attained a value of 30% when expressed conventionally (Leyden *et al.*, 1980) as the percentage reduction in $\log_{10}$ numbers of bacteria: this was statistically significant at $P<0{\cdot}05$. Upon cessation of lotion application the values of recoverable aerobes returned to normal equilibrium levels within 1 week. A similar effect on the aerobic bacteria was seen with the application of the base vehicle, devoid of ethyl lactate.

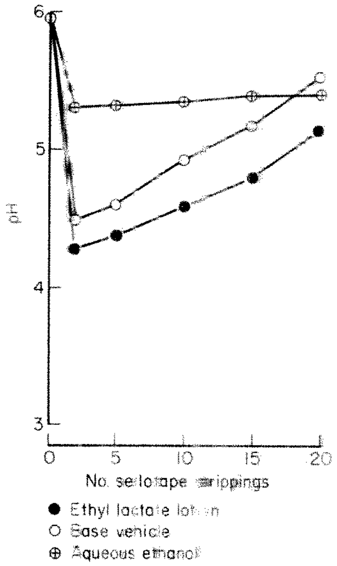

FIGURE 3. Lowering of skin pH throughout the stratum corneum by ethyl lactate lotion. Plotted values are the means of observations upon the treated volar forearms of six volunteers.

For the anaerobic bacteria a greater than 30% reduction in $\log_{10}$ number was seen with the ethyl lactate lotion (significant at $P=0{\cdot}05$) after 2 weeks, whereas the effect of the base vehicle and ethanol-water alone were not significant. This result suggested that the ethyl lactate, by virtue of its accumulation within the pilosebaceous follicles, had a specific effect upon the anaerobes therein. Upon cessation of treatment the recoverable anaerobic bacteria numbers reverted to normal values within 7 days.

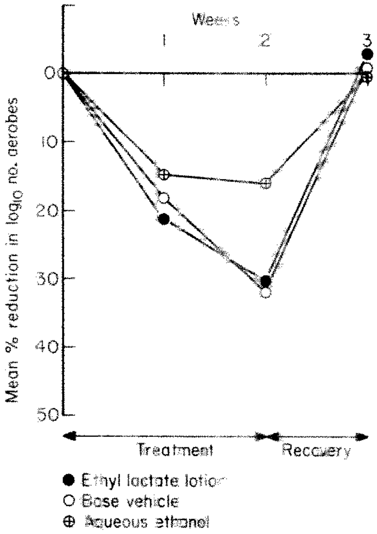

FIGURE 4. Effect of ethyl lactate lotion upon recoverable aerobic bacteria of the skin. Plotted values are the means from eight subjects. Treatments were twice daily for 2 weeks.

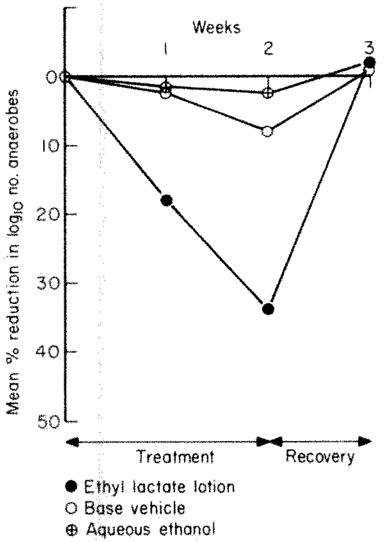

FIGURE 5. Effect of ethyl lactate lotion upon recoverable anaerobic bacteria of the skin. The means of data from the eight subjects used in the experiment of Fig. 4 are plotted.

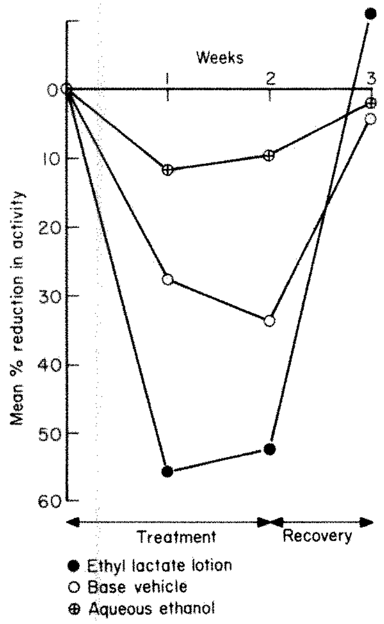

FIGURE 6. Effect of ethyl lactate lotion upon skin surface lipase/esterase activity. Values are the means of data from the same subjects as in Figs 4 and 5.

In a separate experiment the precise levels of *P. acnes* and *P. granulosum* were determined over a 3-week appplication period. The mean percentage $\log_{10}$ reduction at weeks 1, 2 and 3 were 8, 42 and 48 for the former, and 17, 32 and 45 for the latter. This suggests that the ethyl lactate lotion did not preferentially act upon one or other bacterial type.

Effect of ethyl lactate upon sebum hydrolysis

Fig. 6 shows that application of the ethyl lactate lotion caused a greater than 40% reduction ($P < 0{\cdot}01$) in the levels of recoverable skin surface lipase/esterase activity over a 2-week period of daily applications. One week after cessation of application, however, the levels returned to the equilibrium values previously seen before application. The base vehicle (i.e. devoid of ethyl lactate) had a much smaller effect (i.e. approximately 30% reduction in recoverable activity), whereas the ethanol-water vehicle alone had virtually no effect. These findings reflect those of Figs 4 and 5, namely, the reduction in skin bacteria was due to ethyl lactate specifically within the lotion, and it is these micro-organisms that are the likely source of the skin surface lipase/esterase.

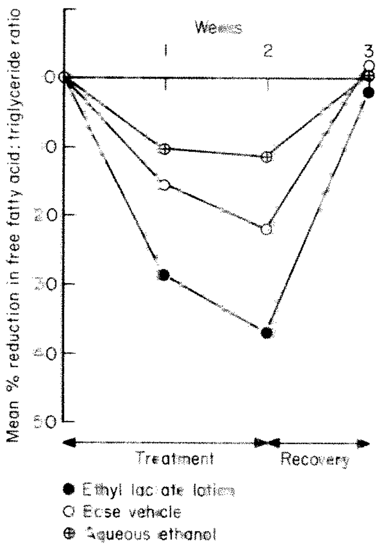

FIGURE 7. Effect of ethyl lactate lotion upon free fatty acid:triglyceride ratios in sebum recovered from the forehead. Values are the means of data from the subjects of Fig. 6.

The consequence of reduced amounts of bacterial lipolytic enzymes should be a reduced hydrolysis of sebum triglycerides to free fatty acids. The confirmation that this occurred is given in Fig. 7. Application to the skin of the ethyl lactate lotion caused between a 30 and 40% reduction ($P < 0{\cdot}01$) in the ratio of free fatty acids to triglycerides in the sebum recovered from the treatment sites. This reduction in the ratio is a direct reflection of reduced triglyceride hydrolysis. After application of the base vehicle, the reduction in the ratio of free fatty acid to triglyceride was much less, and was reduced even further for the aqueous ethanol alone. These data confirmed the specific action of ethyl lactate upon bacterial metabolism of sebum.

DISCUSSION

In 1972 Swanbeck introduced a novel concept for acne treatment utilizing ethyl lactate. It was proposed that topically applied ethyl lactate would be hydrolysed to ethanol and lactic acid in the pilosebaceous duct, and the lactic acid thus generated would lead to a local reduction of pH sufficient to inhibit any further hydrolysis of triglycerides by bacterial lipases to irritant and comedogenic free fatty acids. This mode of action was never properly established, and furthermore Cunliffe & Cotterill (1975) described the disappointing clinical performance of this therapy. More recently, however, Grosshans *et al.* (1978) reported the clinical efficacy of combined treatments of oral tetracycline and topical ethyl lactate lotion, and Zalem & Karovleva (1977) described the effects of ethyl lactate upon facial seborrhoea.

These clinical findings prompted us to study the following questions relevant to the mode of action of ethyl lactate in acne treatment:

(1) Does ethyl lactate penetrate the sebaceous glands?
(2) Does ethyl lactate lower follicular pH, presumably as a result of hydrolysis to lactic acid?
(3) Does ethyl lactate influence the hydrolysis of sebum by bacterial exoenzymes?

We recognized that for each question we were technically constrained as we could only employ indirect measurement procedures for deriving relevant data. For follicular penetration studies we used rats to which ethyl [^{14}C]lactate was topically applied, held under polythene occlusion, and subsequently processed for autoradiography. This approach has been used successfully in the past to investigate skin penetration of materials (Rutherford & Black, 1969), and the current study confirmed the accumulation of radioactivity from the ethyl lactate within the sebaceous glands. These data, which are to be described more fully elsewhere (Black & Howes, in preparation), support the view that ethyl lactate accumulated in the hair follicles and sebaceous glands. From this we extrapolate that this would occur in human skin topically treated with ethyl lactate lotion.

As it is not possible to study follicular events precisely, we resorted to measurements made at the skin surface, which are considered to reflect events occurring within, or originating from the follicles (Cunliffe, 1980). The skin pH studies showed that the ethyl lactate lotion had a marked and additional effect over that of the base vehicle upon surface pH, lowering the equilibrium value by more than 1 unit. Moreover, this remained low for several hours. If this similarly occurred in the pilosebaceous units this could contribute to bacterial enzyme inhibition, and, possibly bacteriostatic or bacteriocidal activity. It is not surprising that the base vehicle had some ability to lower the skin pH, since it was buffered to pH 5·3 with lactic acid and triethanolamine. In separate studies, however, we found it undesirable to lower the skin surface pH much below 4, as this elicited a far higher incidence of astringency and mild 'burning' of the skin.

The tape stripping experiment of Fig. 3 was an attempt to show that as a result of repeated applications of ethyl lactate lotion there was a sustained reduction of low pH within the stratum corneum (i.e. sustained at deeper levels of the skin). This finding supports the contention that sustained 'acidification' of the skin resulted from ethyl lactate application.

The established surface scrubbing method of Williamson & Kligman (1965) was used for sampling skin bacteria as this is reproducible and has been used widely for demonstrating differences between antimicrobial treatments, although we cannot be certain that the upper follicular duct is sampled, or that the anaerobic bacteria obtained are derived from the pilosebaceous units. Leyden *et al.* (1980) have found similar limitations when surface scrub cultures are compared with subsurface cultures of follicular casts obtained by cyanoacrylate glue. Therefore, until better methods are developed for sampling follicular contents, we believe the surface scrub remains the most applicable technique.

Our results showed that with regard to the aerobic bacteria (Fig. 4) the base vehicle was as effective as the ethyl lactate lotion, and this suggests that the ethanol solvent plays a large part in germicidal activity on such bacteria. For the anaerobes however, (Fig. 5), the ethyl lactate lotion was far more effective than the base vehicle, a finding which suggests that ethanol alone is insufficient. Data presented here suggest that ethyl lactate provides the greater germicidal activity by pH reduction of the microenvironment of the anaerobes. In separate studies (unreported) we have grown pure cultures of *P. acnes* in a chemostat. We have found that the maximum yields of the bacterium occurred between pH 5 and 5·4, but at pH 4·3 and below growth ceased completely. Similar findings have been reported by Holland, Cunliffe and Roberts (1978).

The mean reduction in $\log_{10}$ anaerobes after 2 weeks of ethyl lactate application was about 34%: this compares well with similar data reported for 5% benzoyl peroxide (Leyden *et al.*, 1980).

The 'free fatty acid theory' of acne pathogenesis which was considered by Swanbeck (1972) is now thought to be a gross over-simplification of the metabolic changes that elicit inflammatory responses in the acne follicles. Other more complex and numerous products of bacterial metabolism, such as peptides and enzymes such as hyaluronidase, have been identified as important protagonists of the inflammatory response that are chemotaxins, or can trigger the alternative complement pathway (Scott, Cunliffe & Gowland, 1979). However, we consider it likely that as ethyl lactate application has such a marked inhibitory effect upon one aspect of metabolism of the causative microorganisms in acne, by inference, other critical metabolic reactions are probably also inhibited.

Although each of the observations we have made upon the action of ethyl lactate in follicular metabolism is indirect, we would suggest that collectively our data provide a realistic rationale for the clinical efficacy of ethyl lactate in acne vulgaris.

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Comparison of topical clindamycin phosphate, benzoyl peroxide, and a combination of the two for the treatment of acne vulgaris

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SUMMARY

Patients with moderate to severe acne vulgaris were treated for 10 weeks with either topical clindamycin phosphate (1% solution) twice daily, benzoyl peroxide (5% gel) twice daily, or benzoyl peroxide (5% gel) in the morning and clindamycin phosphate (1% solution) in the evening. The effects of each regimen appeared to vary in decreasing specific types of acne lesions, with the combination therapy showing the greatest decrease when all types of lesions were considered. Cutaneous side-effects were greatest with benzoyl peroxide alone during the early weeks of treatment, while the combination therapy displayed no greater incidence of redness, scaling, or itching than clindamycin phosphate alone. All three regimens produced clinical improvements which did not differ significantly from each other.

Various topical antibiotics are effective in acne patients, and their use has now become widely accepted (Stoughton *et al.*, 1980; Stoughton, 1979; Rapaport, Puhvel & Reisner, 1982; Becker *et al.*, 1981; Burke, Eady & Cunliffe, 1983).

Topical clindamycin phosphate, a water-soluble ester, appears to be more effective than either topically applied erythromycin or tetracycline in lowering counts of *Propionibacterium acnes* on the skin (Stoughton & Resh, 1976; Resh & Stoughton, 1976). While effective in treating acne, topical clindamycin does not result in measurable blood levels (Becker *et al.*, 1981). Clindamycin has also been noted to inhibit free fatty acid production which is considered to be a possible mediator of the inflammatory mechanism (Leyden *et al.*, 1980).

Another widely used topical preparation for acne therapy is benzoyl peroxide. It is an antibacterial agent to which microbial resistance cannot ordinarily be induced (Rapaport & Puhvel, 1983). It is effective in causing desquamation and suppressing levels of free fatty acids in the sebum. However, in clinical investigations, it has also been found to be a sensitizer on the order of 1–2% (Lyons, 1978).

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In this study, three regimens were randomly assigned and compared to assess the benefit of therapy with benzoyl peroxide (5% gel), topical clindamycin phosphate (1% solution), and a combination of these products in patients with acne vulgaris. The rationale for this study stems from the frequent combination of these agents for the treatment of patients with acne (Burke *et al.*, 1983; Rapaport & Puhvel, 1983).

METHODS

Male and female patients between 12 and 30 years of age with moderate to severe acne vulgaris were studied. All patients had between twelve and seventy inflammatory lesions, but no more than ten nodulocystic lesions located at or above the jawline. A nodulocystic lesion was considered an indurated inflammatory lesion measuring at least 8 mm or greater. Exclusion criteria included patients with chronic bowel disease or frequent, periodic diarrhoea as well as patients receiving systemic antibiotics, steroids, or androgenic drugs within the previous 30 days, or topical medication for acne within the last 14 days.

Seventy-nine patients were enrolled in the study and divided into therapy groups as noted in Table 1. Individuals were provided with boxes containing a non-medicated, mild soap as well as the topical medicines at bi-weekly visits. After each visit, the previous samples were returned for weighing to determine the amount of medication consumed. The investigators were not informed as to which treatment group the patients belonged, but the medication given to each patient was in a commercial package with the label still intact. Two weeks before the start of the study, all acne medication was discontinued and the subjects used only a non-medicated, mild soap for therapy.

TABLE 1. Distribution of patients among treatment groups by sex

Treatment	Male	Female	Total
Clindamycin phosphate (1% solution)	15	14	29
Benzoyl peroxide (5% gel)	10	16	26
Combination of therapy	9	15	24
Total	34	45	79

Patients were instructed to use the medication twice daily (clindamycin phosphate alone, benzoyl peroxide alone, or benzoyl peroxide in the morning and clindamycin phosphate in the evening). Evaluations of these treatment regimens were made at each bi-weekly visit. At this time, an acne score was assigned based on a scale of 1–4, with: 1 = numerous comedones, some papules; 2 = papules and some pustules; 3 = papules, pustules, and occasional cysts; and 4 = primarily cystic acne. Evaluations of irritancy included the examiners' recording of erythema, peeling, dryness, burning, and itching by applying a scale of 0–3, with: 0 = none; 1 = mild; 2 = moderate; and, 3 = severe. An index of tolerance to the medication was calculated by combining the irritancy scores recorded at each visit. Counts of papules, pustules, comedones, and nodulocystic lesions were also recorded at each visit. A severity index was then developed by multiplying the number of each specific type of lesion by a weighting factor as follows: comedo = 0·25; papule = 1·0; pustule = 2·0; and nodulocyst = 3·0 (Michaelsson, Juhlin

& Vahlquist, 1977). Side-effects were evaluated at each visit with specific questions asked about symptoms of diarrhoea. A urine specimen was collected on the 2nd and 8th week of therapy and analysed for the concentration of clindamycin HCl.

Statistical analyses used an analysis of variance (ANOVA), with repeated measures, for the evaluation of the severity index. Differences at individual time points between treatment means were determined through the application of the least significant difference (LSD) multiple comparison test. Fifty-six of the patients completed the full course of therapy. Three patients withdrew due to a lack of efficacy. The overall rate of dropout across the three treatment groups was comparable, therefore comparisons were made only on patients who actually completed the full investigation.

RESULTS

The effect of the various treatment regimens by type of lesion is shown in Figure 1. All treatments lowered the number of comedones, papules, pustules, and cysts by the end of the 10-week period. No specific treatment was found to be significantly better in all categories. While significant differences did occur throughout the study between certain treatment groups

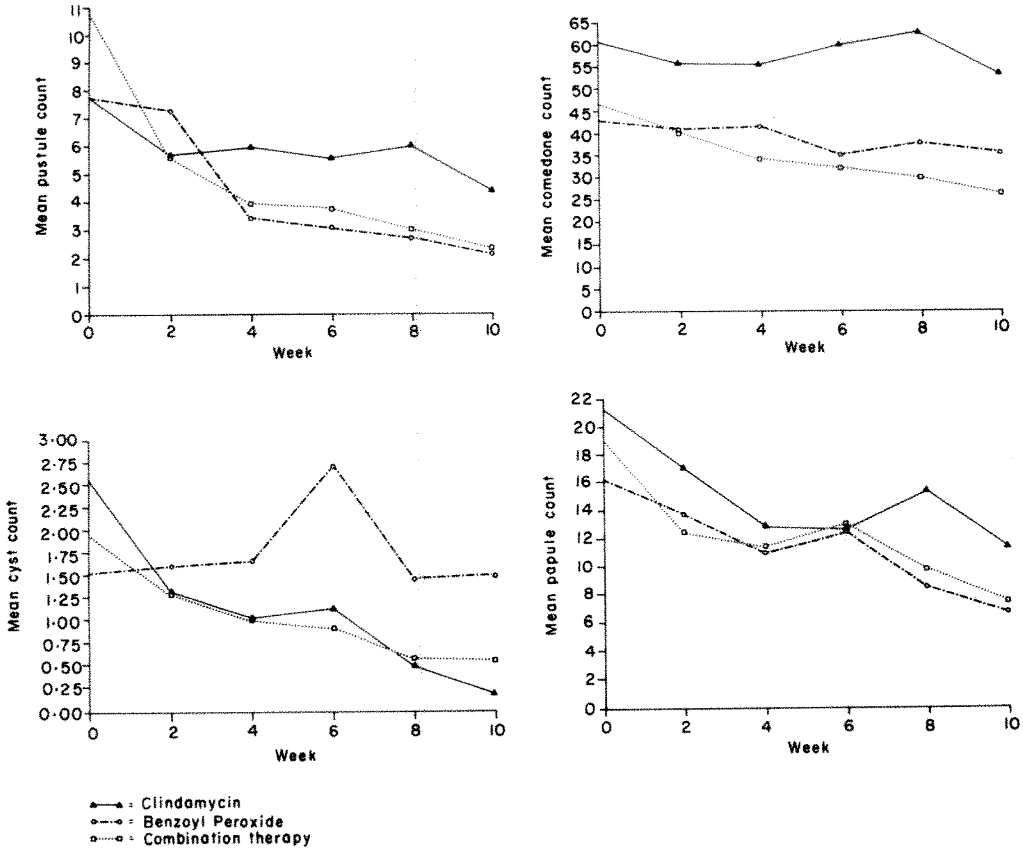

FIGURE 1. Response of individual types of lesions to the three different treatment regimens during the 10-week evaluation period.

and specific types of lesions, no consistent pattern was apparent. The acne severity index (Fig. 2) shows that the combination therapy gives consistently lower severity scores throughout the evaluation period. There was no statistically significant difference in the severity index between treatment groups. However, all groups did display a lower severity index by week 10 as compared with week 0 ($P \leq 0{\cdot}05$).

The irritancy index indicates that benzoyl peroxide had a significantly higher score than either clindamycin phosphate alone or the combination therapy ($P \leq 0{\cdot}01$). Note that the combination therapy, which utilized an application of each medication daily, did not give an intermediate irritancy score, but was similar to clindamycin phosphate alone. Comparing the irritancy index (Fig. 2, right) with cyst count (Fig. 1), a possible relationship becomes apparent. At week 2, a marked degree of irritancy was evident with cystic lesions gradually increasing and peaking by week 6 for benzoyl peroxide. This contrasts sharply with the decrease in cysts that was seen for clindamycin phosphate and the combination therapy.

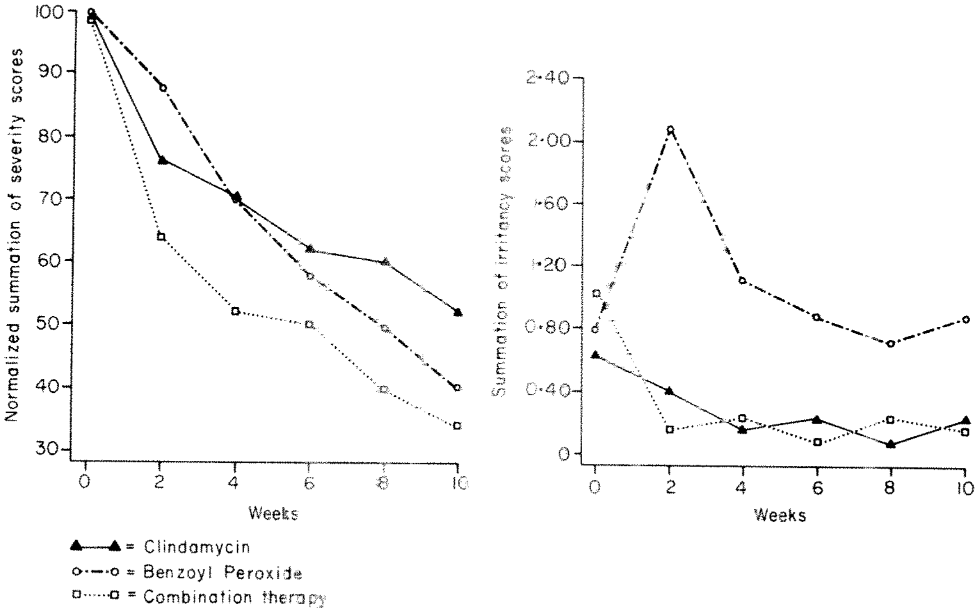

FIGURE 2. Acne severity index (left) and irritancy index (right).

The rate of dropout in the three treatment groups was comparable, although only in the benzoyl peroxide group did three participants withdraw due to treatment failure. A total of eight patients reported diarrhoea at some point during the study but no statistically significant difference was found for this side-effect between the treatment groups. The subjective rating of the investigators (based on a scale of 1–4) indicated an improvement of acne by all three treatment regimens. At the completion of the study 95%, 82%, and 96% of the patients using clindamycin phosphate, benzoyl peroxide, and the combination therapy respectively, felt their acne had improved. No statistical difference was noted in the subjective rating between the three treatment groups. Urine samples analysed for antimicrobial activity were all negative at a minimum detectable limit of 0·25 μg/ml for clindamycin HCl.

DISCUSSION

Topical antibiotics have become a standard therapeutic regimen for patients with acne vulgaris, and this study further substantiates the efficacy of these agents. The combination of therapy involving clindamycin phosphate (1% solution) in the morning and benzoyl peroxide (5% gel) at night consistently demonstrated the best overall efficacy when the acne severity index is applied. Irritation with this combination of therapy was not greater than that with clindamycin phosphate alone and less than that with benzoyl peroxide alone. This may be of considerable importance as benzoyl peroxide is known to be a sensitizing agent; a property that may be enhanced in the presence of cutaneous irritation (Poole, Griffith & MacMillan, 1970; Rostenberg, 1957). A previous study reported a decrease in irritancy when benzoyl peroxide was used in combination with topical retinoic acid, which supports our present clinical findings (Hurwitz, 1976).

A problem of developing bacterial resistance to individual topical antibiotics has also been reported (Crawford *et al.*, 1979). A recent study on a combination of topical antibiotics, one of which was benzoyl peroxide, showed an absence of this phenomenon for *Propionibacterium acnes* (Rapaport & Puhvel, 1983). Benzoyl peroxide alone does not ordinarily allow an emergence of resistant organisms, suggesting that its use may have been beneficial in retarding bacterial resistance (Kligman, Leyden & Stewart, 1977). Benzoyl peroxide also possesses keratolytic properties that are beneficial in the treatment of acne (Fulton, Farzad-Bakshandeh & Bradely, 1974).

Clindamycin has been reported to possess anti-chemotactic activity (Esterly, Furey & Flanagan, 1978). Since the attraction of cells may be an important aspect in the inflammation of acne vulgaris, the effectiveness seen with topical antibiotics may be, in part, accounted for by this property (Tucker *et al.*, 1980). The combination of the two antibiotics topically applied to the skin may be synergistic due to the differing mechanisms by which they appear to improve the acne lesions.

Topical antibiotics have assumed a major role in the treatment of acne vulgaris. This study supports the use of a combination of clindamycin phosphate and benzoyl peroxide to prevent irritation and to broaden the therapeutic spectrum by using agents with different mechanisms of action which are effective against different types of acne lesions.

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Brief Communication

Suppression of Behçet's disease with dapsone

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SUMMARY

Seven male patients with Behçet's disease were treated with dapsone 100 mg daily. All had suppression of their pathergic reaction to a deep needle-prick, with noticeable symptomatic improvement.

Various drugs have been used to treat Behçet's disease, e.g. corticosteroids (O'Duffy, Carney & Deodhar, 1971), immunosuppressive agents (Abdalla & Bahgat, 1973), levamisole (de Merieux, Spitler & Paulas, 1981) and colchicine (Miyachi *et al.*, 1981). Dapsone has not to our knowledge been tried in Behçet's disease but it has beneficial effects in several other dermatological disorders including dermatitis herpetiformis, subcorneal pustular dermatosis and erythema elevatum diutinum (Lang, 1979).

METHODS

Seven male patients with complete Behçet's disease were studied. The age of onset of the disease ranged from 21 to 44 years. They all had oro-genital ulcerations and erythema nodosum-like lesions; four of them had ocular lesions and arthritis, and five patients had orchitis. Furunculosis was observed in three patients. All patients had a positive pathergic reaction to intradermal needle-prick before therapy. The patients were given dapsone 100 mg a day for 4–7 months and watched carefully for any change in their symptoms. The prick test was repeated within a few days of initiation of therapy and also when dapsone was discontinued for some days during the course of treatment.

RESULTS

After a few days of treatment with dapsone, the pathergic test (prick test) was repeated and it was completely negative in two patients while it diminished greatly in size in the others. The test was found to be slightly positive in all patients when repeated again during the course of therapy. It again became strongly positive when dapsone was stopped for a few days.

Dapsone had a beneficial effect in all patients. The oro-genital ulcers, erythema nodosum and boil-like lesions cleared up within a few weeks. A few patients continued to develop oro-genital ulcers during the course of treatment, but these were infrequent, few in number, smaller, much less painful and healed quickly. Two patients flared up on stopping dapsone so treatment was resumed. No side-effects from dapsone were noted in these patients.

DISCUSSION

The pathogenesis of the pathergic reaction is largely accounted for by a polymorphonuclear cell infiltrate, at least in the first 24 h. Subsequently the mononuclear cells predominate (Izuno & Kazuyuki, 1978). This reaction may reflect the increased chemotaxis of polymorphonuclear cells that has been shown in Behçet's disease (Fordham *et al.*, 1982). In the present study, this reaction was suppressed by dapsone, presumably by inhibition of chemotaxis of polymorphonuclear leukocytes (Anderson *et al.*, 1981). The mechanism of the beneficial effect of dapsone in Behçet's disease, as well as in various other dermatoses in which polymorphonuclear leukocytes play a major role, might be explained by the inhibition of lysosomal activity and interference with the myeloperoxidase H_2O_2–halide mediated cytotoxic system in polymorphonuclear leukocytes (Stendahl, Molin & Dahlgren, 1978). It has also been reported that dapsone exerts some of its anti-inflammatory effects by interfering with polymorphonuclear leukocyte-dependent production of oxygen intermediates, thus conferring protection from auto-oxidative tissue injury (Miyachi & Niwa, 1982).

The clinical response to dapsone now needs to be confirmed by a carefully controlled trial.

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Case Report

Autoimmune haemolytic anaemia occurring in a patient with mycosis fungoides

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SUMMARY

We report a case of the association of Coombs'-positive haemolytic anaemia with mycosis fungoides. The patient was found to have polyclonal elevation of B lymphocytes in the peripheral blood but infiltration by T helper cells in the skin.

Autoimmune, Coombs'-positive, haemolytic anaemia has a well-known association with malignant B-cell lymphoma. However, we are not aware of it having been reported in association with a cutaneous T-cell lymphoma.

CASE REPORT

A man now aged 76 years first presented to this department 4 years ago with a 1-year history of gradually developing itchy scaly plaques initially on the legs and spreading to the rest of the body. The clinical picture was consistent with a diagnosis of plaque stage mycosis fungoides.

Histological examination of a skin biopsy revealed a dense upper dermal infiltrate of lymphocytes and histiocytes. Cells with large multinucleated or lobed nuclei were scattered diffusely in small numbers in the papillary dermis and foci of mononuclear cells were seen within spaces in the epidermis (Pautrier micro-abscesses). Biopsy of an axillary lymph node performed at that time showed changes consistent with dermatopathic lymphadenopathy and a liver biopsy showed fatty change only. Full blood count was normal but typing of the blood lymphocytes showed a reversal of the usual T/B cell ratio, with T cells 9% and B cells 80% and a similar ratio in the marrow, which was otherwise normal. Because of the limited techniques available at that time this was taken no further and it was not known whether this was a monoclonal or polyclonal rise in the B-cell population. It was presumed that the dermatological diagnosis was mycosis fungoides and treatment with photochemotherapy (PUVA) was given but was ineffective. Subsequent electron beam therapy for 24 weeks cleared all lesions.

He remained well until 18 months ago when he was found to be anaemic with a haemoglobin of 8·1 g/dl, white cell count of $6{\cdot}8 \times 10^9/l$ and platelet count of $300 \times 10^9/l$; the reticulocyte count was 17·4% and the direct Coombs' test was strongly positive, due to complement binding to

IgG_1 and IgG_3, on erythrocytes. The antibody was found in the serum but the specificity was not to any known blood group antigen. There were increases in the levels of IgG (17·0, normal 9·5–16·5) and of IgA (6·8, normal 0·7–4·5). Autoantibody screen was negative. He had been taking no drugs throughout his illness apart from 8-methoxypsoralen 2 h pre-PUVA, and pivmecillinam hydrochloride for 2 weeks during his first admission because he was found to be a salmonella carrier. He was started on 30 mg of oral prednisolone daily and within 2 weeks his haemoglobin had risen to 10·4 g/dl, and by 6 weeks to 14·1 g/dl. Prednisolone ws reduced to 20 mg per day.

Results of further investigations on the blood are shown in Table 1. The markers of total T cells (OKT3 positive) and suppressor phenotype (OKT8 positive) were normal. There was an increase of B cells (SmIg positive). The B cells appear to be polyclonal since kappa and lambda bearing cells were both increased, as were IgM bearing cells. Monoclonal antibody studies were performed on two full thickness skin biopsies from involved skin from the forearm and thigh. Fresh cut cryostat sections were examined with the following mouse monoclonal antibodies: OKT3, OKT4, OKT8, OKT6, OKM1 (Ortho Diagnostics) I2, B1 (Coulter Electronics), common leucocyte antigen T200 (Hybritech Incorporated). Histochemically the tumour cells reacted with OKT3, OKT4 and CLAT 200 monoclonal antibodies, indicating a T helper derivation. A few Langerhans cells (OKT6) and macrophages (OKM1 and I2) were associated with the epidermal microabscesses. No B cells were demonstrated in the biopsy specimens.

DISCUSSION

It is now widely accepted that mycosis fungoides and the Sézary syndrome are part of a broader spectrum of T-cell lymphomas (Edelson, 1980) and to date the only group of adult lymphoproliferative disorders regularly demonstrated to be of T-cell origin are the cutaneous T-cell lymphomas (Edelson *et al.*, 1974).

TABLE 1.

Blood lymphocytes % of white cells		2·18 × 10^9/l 24		Normal range × 10^9/l 1·0–3·0
		(%)	(× 10^9/l)	
T cells				
Sheep RBC rosettes		63	1·37	0·6–2·2
Monoclonal anti T-rosettes (OKT3)		65	1·42	0·6–2·2
OKT8 positive		37	0·81	
B cells				
Surface immunoglobulin positive		31	0·68	0·1–0·5
Surface membrane				
Immunoglobulin class	IgM	30	0·65	0·06–0·2
	IgD	24	0·52	0·08–0·27
	IgG	2	0·04	0·09–0·29
	IgA	1	0·02	0·02–0·09
Light chains				
Kappa		21	0·46	0·08–0·26
Lambda		10	0·22	0·04–0·13

The association of autoimmune haemolytic anaemia and lymphoproliferative disorders is well established, occurring with a range of conditions including plasma cell dyscrasias, the leukaemias (particularly chronic lymphocytic leukaemia), and lymphomas; these are all known to be tumours of B-cell origin (Gerard-Marchant *et al.*, 1974).

Two large studies of patients with mycosis fungoides have not shown evidence of autoimmune haemolytic anaemia (Cyr *et al.*, 1966, Mycosis Fungoides Cooperative Study Group, 1975). There is no mention either of mycosis fungoides or Sézary syndrome in Jones' (1973) review of autoimmune disorders and malignant lymphomas. It may seem paradoxical to have a condition usually associated with B-cell lymphoproliferation occurring in a T-cell cutaneous lymphoma. However, evidence of increased B-cell activity has been demonstrated by the findings of an elevated IgE level in one series of fourteen patients (Mackie *et al.*, 1976) and a report of the finding of a monoclonal IgA gammopathy in one patient (Joyner *et al.*, 1979) as well as the occurrence of a monoclonal IgG lambda gammopathy in one of our own (Lawrence *et al.*, in preparation). At the time of development of his anaemia our patient had a slightly raised IgG and a moderately raised IgA level and latterly, a large increase in surface membrane IgM and IgD on blood lymphocytes (Table 1). The finding of a polyclonal elevation of the B-cells is against his having a second malignant lymphoproliferative disorder.

We therefore postulate that there is a possible functional sequel to the T helper-cell proliferation. It is known that T helper-cells play a role in immunoglobulin production (Katz & Benacerrat, 1979) and it would be reasonable to suppose that they could induce several clones of plasma cells, one of which may have produced the haemolytic antibody.

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Comment

IMMUNOELECTRON MICROSCOPY—HOW USEFUL IS IT FOR THE DERMATOLOGIST?

Immunoelectron microscopy is a research tool that has been developed from immunomorphological techniques such as immunofluorescence, which were originally designed for the light microscope. This development allows comparable immunological methods to be used at higher levels of magnification in the electron microscope.

Due to inherent technical requirements other labels have had to be employed. Whereas immunofluorescence may utilize low molecular weight labels (e.g. fluorescein or rhodamine) electron microscopy must resort to substances of higher electron density to ensure visibility on the electron screen, e.g. metals or enzyme reaction products. Various possible techniques have now been elaborated (Albini *et al.*, 1979; Holubar & Wolff, 1983). Today the most widely used marker in immunoelectron microscopy is horseradish peroxidase, bound covalently or immunologically to immunoglobulin molecules which, in turn, serve as either antibodies or antigens (Boorsma, 1977; Heyderman, 1979). Polyclonal and monoclonal antibodies are used. (Horseradish peroxidase is readily available; sensitive techniques have been developed and the reaction product is clearly visible if benzidine-type substrates are employed.)

Techniques have also been developed to allow paraffin-embedded tissue to be successfully subjected to immunological reactions (Heyderman, 1979), but for immunoelectron microscopy it is still most desirable to use native unfixed or prefixed tissue, and except for special purposes, tissue should not be subjected to freezing and thawing, to prevent disruption of membrane structures and cell organelles.

The same basic techniques that are used to prepare tissue for the light microscope are also applied to the incubation procedures in immunoelectron microscopy, though the latter is a more costly, time-consuming and delicate procedure. Many laboratories have accumulated enough expertise to use immunoelectron microscopy as a routine procedure and it is readily available in many centres.

It is beyond the scope of this article to enter into the technical details of all the different incubation procedures which have been described and refined over the last decade. These include: peroxidase–antiperoxidase (PAP) methods with newly formed or preformed complexes (Sternberger *et al.*, 1970; Holubar *et al.*, 1975; Sternberger & Petrali, 1977; Sternberger & Joseph, 1979); double PAP methods (Ordronneau, Lindström & Petrusz, 1981); other double incubation techniques (Vacca, Abrahams & Naftchi, 1980); 'mixed' methods employing different types of labels (van Rooijen 1980); and the labelled antigen and similar methods may be mentioned (Mason & Sammons, 1979; Tougard, Tixier-Vidal & Avrameas, 1979; Holden & MacDonald, 1983). In addition, several other methods have been devised which, strictly speaking, are not immunological methods in the classical sense of the term (see below). Furthermore, the development of the hybridoma technique and the tailor-made production of monoclonal antibodies has revolutionized the field (Milstein, 1980; Milstein, 1982; Berger & Edelson, 1982). Protein A of staphylococcal origin for the detection of IgG, the avidin–biotin system (as a non-immunological tracer system) and lectins for the detection of certain

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carbohydrate moieties, may be listed as examples (Bayer & Wilchek, 1978; Bayer, Rivnay & Skutelsky, 1979; Celio, 1979; Hsu & Raine, 1981; Notani, Parsons & Erlandsen, 1979; Sharon, 1977; Trost *et al.*, 1980). These non-immunological techniques may be combined with classical (immunological) ones (Naritoku & Taylor, 1982).

What is the current practical value of immunoelectron microscopy for the clinical dermatologist? When should he ask to have this technique employed, and what may he expect as an answer?

Immunoelectron microscopy is not a screening technique.

It should preferably be performed only if a specific question can be posed or if previously employed techniques have failed to solve the problem.

Closed (as opposed to open-ended) questions should be asked, e.g. 'Is it *a* or *b*?' and not 'What is it?'

Results must be evaluated in the light of the results from other investigations.

The following questions can be answered by application of immunoelectron microscopy.

(1) Is it a form of bullous pemphigoid, i.e. is there a deposition of Ig or complement (C3) in the lamina lucida? (Holubar *et al.*, 1975; Schmidt-Ulrich *et al.*, 1975; Holubar, Konrad & Stingl, 1977; Hönigsmann *et al.*, 1976)*

(2) Is it pemphigus or one of its variants? (Chorzelski *et al.*, 1968; Wolff & Schreiner, 1971).

(3) Is it (bullous) lupus erythematosus, i.e. is there a deposition of Ig distributed irregularly over the junctional zone? This can be a useful way to distinguish the condition from bullous pemphigoid (Pehamberger, Konrad & Holubar, 1978; Olansky *et al.*, 1982).

(4) Is it epidermolysis bullosa acquisita or one of the forms of congenital epidermolysis?* The definitive diagnosis of the different forms of congenital epidermolysis can be made either by conventional electron microscopy or by so-called 'antigen mapping' (Hintner *et al.*, 1981; Nieboer *et al.*, 1980; Yaoita *et al.*, 1981). 'Antigen Mapping' is a procedure of topographical localization of antigens, for instance in the junctional zone of skin, and their relation to the level of cleavage in blistering diseases and to one another. The localization of bullous pemphigoid antigen, laminin and type IV collagen relative to the level of cleavage, allows the differentiation between epidermolytic, junctional and dermolytic bullae.

(5) Is it dermatitis herpetiformis, i.e. is there a deposition of IgA below the basal lamina? (Yaoita & Katz, 1976; Stingl *et al.*, 1976; Katz & Lawley, 1979).

(6) Is it chronic bullous disease of childhood, i.e. do we find a linear deposition of IgA along the basement zone by immunofluorescence, and a deposition of IgA in the lamina lucida by immunoelectron microscopy? The latter finding constitutes the result of investigations in one clinically typical case (Holubar & Wolff, unpublished).*

(7) What different classes of lymphocytes are present, i.e. what are the functional subtypes as identified by their surface characteristics? (Kung *et al.*, 1983). This can be an important

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* The above questions may be answered on the basis of the morphological results obtained, e.g. deposition of certain substances, for instance IgG or IgA, in or below the lamina basalis, in the lamina lucida or in the surface coat of keratinocytes. Such a morphological finding must then be interpreted for its relevance to a given disease in accordance with current diagnostic criteria. It cannot be stated, for instance, that the deposition of IgG below the lamina basalis in epidermolysis bullosa acquisita, is a single criterion that will create an entity on its own. In the given example, it must therefore remain open whether such a type of blistering disease is a special variant of bullous pemphigoid or whether it belongs to another group or whether it is an independent entity. The same holds true for chronic bullous disease of childhood, and the various subsets of dermatitis herpetiformis. Only the identification of the responsible antigens(s) will allow us the draw more reliable conclusions.

contribution to the differential diagnosis of lymphoma, provided the surface markers are expressed.

(8) Are Langerhans and histiocytosis X cells present? (Wolff & Stingl, 1983; Schuler *et al.*, 1983; Bronstein *et al.*, 1983; Murphy *et al.*, 1983).

(9) What is the ontogenetic origin of cells (ectodermal vs. mesodermal vs. entodermal etc.)? This can be achieved by identification of subcellular structures such as keratin filaments, vimentin, desmin, neuro- and glial filaments. Also, the degree of differentiation of cells can be assessed (Ramaekers *et al.*, 1983; Hintner *et al.*, 1983).

(10) What is the localization of different antigens which appear at the 'same' site by light microscopy? It has been shown that the lamina lucida harbours bullous pemphigoid antigen, laminin and a proteoglycan substance (Stanley *et al.*, 1982) and that the lamina basalis contains type IV collagen and another specific antigen (Breathnach *et al.*, 1983).

It will be apparent from the above that immunoelectron microscopy offers the greatest aid in the differential diagnosis of bullous eruptions and in the diagnosis of lymphomas and leukaemias.

Immunoelectron microscopy yields only morphological data and it suffers from the same limitations as other methods of immunomorphology, e.g. immunofluorescence. Evaluation can be made on such a morphological basis but will have to remain restricted until more information about the nature of the demonstrated target is available. On the other hand, data obtained by functional assays can be supplemented by immunoelectron microscopy. Particularly useful information can be gained about the localization of the reactants inside a cell, in a cell organelle and in or along the cell membrane.

With the application of such sophisticated reagents as monoclonal antibodies it must be expected that even with electron microscopy, more than one antigen will be found to occur in the 'same' place (Breathnach *et al.*, 1983). Localization *and* identification of the targets (antigens) will both be needed to enable us to draw better conclusions as to their respective roles *in vivo* and *in vitro*.

Immunoelectron microscopy does not invalidate any other technique, particularly not immunofluorescence. In accordance with what has been outlined above about the cost in time, manpower and equipment, immunoelectron microscopy will remain restricted to large centres, and even there it will be governed by economic considerations. Undoubtedly, however, the rapid developments in the fields of immunology and electron microscopy will bring about wider and even more specific applications of this useful technique.

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British Journal of Dermatology (1984) **110**, 504–506.

Correspondence

Granulocytic spongiotic papulovesiculosis. A new entity?

SIR, There are several papular inflammatory dermatoses that preferentially involve the anterior chest, e.g. transient acantholytic dermatosis (Grover & Park, 1970) and prurigo pigmentosa (Nagashima, 1978; Cotterill & Greenwood, 1981). We here describe a boy suffering from pruritic, corticosteroid-resistant, dapsone-responsive, papulovesicles on the upper trunk and neck with granulocytic spongiosis, and we suggest that this may constitute a new entity.

A 15-year-old boy, who had been treated with chlorpromazine and promethazine for schizophrenia for 3 weeks, was referred to us with a 1-week history of a pruritic, papular eruption on the upper part of the trunk and the neck.

On examination, there were small tense vesicles scattered among a papular rash spreading over the trunk and neck (Fig. 1). Results of routine laboratory studies were within normal limits, except for slight eosinophilia (9%).

A biopsy of one of the vesicles showed marked spongiosis in the epidermis. This coalesced to form intraepidermal abscesses and a dense perivascular infiltration of mononuclear cells in the upper dermis (Fig. 2). The intraepidermal abscesses were filled with neutrophils and eosinophils. No findings suggestive of vasculitis were seen in the dermis. Direct immunofluorescence studies did not reveal any specific deposits of IgG, IgM, IgA, C1q, C3 or fibrinogen at the intercellular portion of the epidermis, dermo-epidermal junction or perivascular portion of the dermis. Indirect immunofluorescence was also negative for auto-antibodies against skin components.

Because of our failure to suppress the eruption by oral corticosteroids (i.e. prednisolone 10 mg daily for 1 week, and methylprednisolone 12 to 16 mg daily for an additional 2 weeks), we started administration of 75

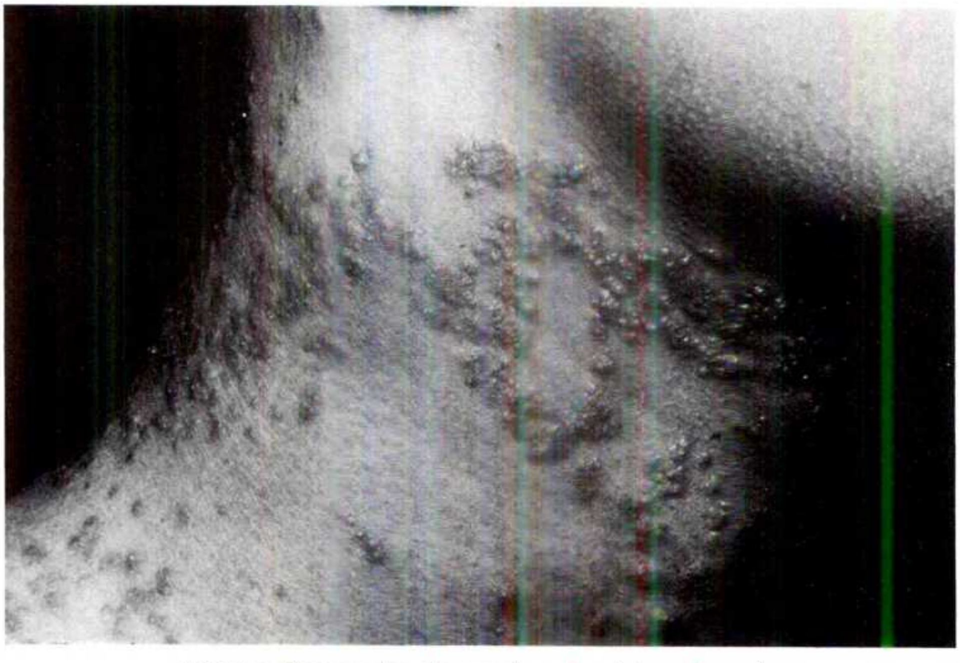

FIGURE 1. Close-up view of grouped papulovesicles on the neck.

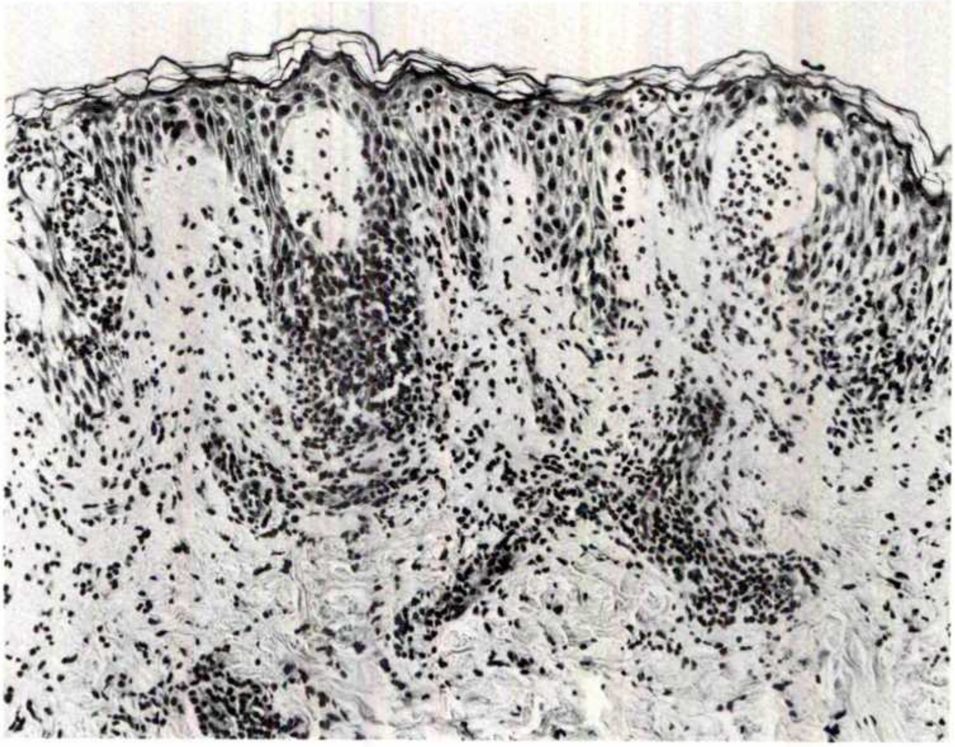

FIGURE 2. Marked spongiosis with intraepidermal microabscesses containing neutrophils and eosinophils and a dense perivascular cellular infiltrate (H & E, × 40).

mg of dapsone daily. One week later, the eruption was remarkably alleviated. The dose of dapsone was gradually diminished to zero over the following 3 weeks, and there was steady resolution of the lesions. Although the eruption recurred once on the anterior chest 4 months later, again without any apparent cause, it subsided 2 days after readministration of 75 mg of dapsone daily.

This patient presented a unique combination of clinical and histological features which did not show any apparent relation to previously reported dermatoses. The clinical features were characterized by rapidly progressing, pruritic papulovesicles on the trunk and the neck, which were not greatly influenced by a moderate dose of oral corticosteroids, but did respond well to dapsone. Though the eruption appeared 3 weeks after starting chlorpromazine and promethazine, a vesiculopustular drug eruption is unlikely since the skin lesions quickly disappeared following dapsone therapy, despite the continuation of these drugs. Furthermore its recurrence occurred independently of these drugs.

The prominent histological findings were marked spongiosis with intraepidermal microabscesses filled with neutrophils and eosinophils, which is similar to the findings in eosinophilic spongiosis in pemphigus (Emmerson & Wilson-Jones, 1968). Such changes may also be considered compatible with those of contact dermatitis, particularly of primary irritant type. However, the clinical appearance, with discrete papulovesicles, and the ineffectiveness of both topical and systemic steroids argue against that diagnosis. No contact irritants or allergens could be detected, despite our repeated questions.

Although our case showed some similarities to previously described clinical entities such as pemphigus with eosinophilic spongiosis, dermatitis herpetiformis, prurigo pigmentosa and transient acantholytic dermatosis, differential diagnosis was easily made from them, based on the clinical, histological and immunohistological findings. Thus we tentatively propose the term 'granulocytic spongiotic papulovesiculosis' for this condition.

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Topical benzoyl peroxide increases the sebum excretion rate

SIR, We read with much interest the paper on topical benzoyl peroxide by Cunliffe, Stainton and Forster (1983). We would like to report a similar conclusion after using benzoyl peroxide 2·5, 5 and 10% during a period of 1 year in eighty-seven patients with acne. Our data (Pierard-Franchimont, Melotte & Pierard, 1983) were obtained by measuring monthly the sebum excretion rate with the Lipometre instrument (L'Oréal). In our experience, topical benzoyl peroxide produces a significant rise in the sebum excretion rate as early as the second month of treatment. After a continuous elevation in SER, a new stable value, increased by about 45% as compared with the pretreatment value, is gained for SER when lesions of acne vulgaris are improved. No patient complained about these changes in sebum excretion.

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British Journal of Dermatology (1984) **110,** 507–509.

Book Reviews

Fundamentals of Dermatology. T.NASEMANN, W.SAUERBREY AND W.H.C.BURGDORF (1983) Heidelberg: Springer. Pp. 379. Price DM 68.00.

Professor Walter Burgdorf explains in the preface that this book is a translation of Professor Nasemann's original German text which has been very successful and is in its fourth edition. New sections have been added on Tropical Dermatology and Cutaneous Surgery and the therapy sections have been revised to incorporate current practice in the U.S.A.

The book lives up to its title in that it provides a systematic, clear, and concise approach to the diagnosis and management of dermatological diseases. I think nothing has been lost in the translation and indeed Professor Nasemann's precise description of the clinical features shines through. The text is illustrated with well-chosen black-and-white photographs. There was only one which I think could be improved and that was the illustration of discoid lupus erythematosus on page 169 which does not show clear follicular plugging, and the widespread involvement of the lower lip is confusing. The tables, diagrams and differential diagnoses are clear and very helpful. There is throughout the book a great attention to detail both in the description of clinical signs and the investigations which should be done to confirm a diagnosis; this is well illustrated by the advice given in the mycology section to use an old microscope when looking for fungus. There are one or two curiosities which authenticate the text. To British dermatologists the detailed description of the Auspitz sign in psoriasis, which involves removing scale until bleeding points appear, is probably unnecessary. We would also be doubtful about the value of an elimination diet of oatmeal tea and Zwiebach for several days, although we wish that it would sort out the ever-present problem of patients with chronic urticaria.

The advice on treatment in the text and summarised in Appendix I is up to date and comprehensive. There are many helpful suggestions and only in one area I think would there be major disagreement and that is in the use of potent topical corticosteroids in the management of psoriasis, particularly when pustular or progressing to erythroderma.

At the end of the book the list of abbreviations is welcome because there are now so many that they can be very confusing. The colour plates covering certain important areas of dermatological differential diagnoses are of excellent quality. Finally, the bibliography compiled by Walter M.C.Burgdorf with personal comments is a novel but stimulating way of introducing the reader to most of the standard dermatological references. Although there are many medium-sized dermatological textbooks on the market I think this is a useful addition. It is clearly written and should give the undergraduate and those in training a systematic approach to dermatological diagnosis. In addition I think it will also be of interest to general physicians and established dermatologists.

G.W.BEVERIDGE

Biochemistry and Physiology of the Skin. Edited by L.A.GOLDSMITH (1983) New York: Oxford University Press. Two volumes, Pp. 1323. Price £79.00.

It is almost 30 years since Rothman wrote his classic textbook on skin physiology and biochemistry, and there is clearly a need for an updated work to provide a comprehensive review of this huge field. Goldsmith and his international team of eighty-five experts, many of whom have made major contributions to the original literature, have now made a gallant attempt to supply this need. Their book will undoubtedly provide a useful reference source, and will probably be a commercial success, but it does not to my mind provide the useful overview of the subject that Rothman produced.

Dr Goldsmith faced an impossible task in co-ordinating the contributors to this huge work, which tackles anatomy, physiology, biochemistry, pharmacology, immunology, cell kinetics, wound healing, effects of irradiation, biophysical properties, and if that were not enough, the pathophysiology of blistering, carcinogenesis, infections and nutritional defects. The information may all be in there somewhere, but it is certainly not easy to extract. The Merkel cell provides a typical example of the way in which the book fails to provide a clear and coherent account of some of the topics covered. Although these cells are discussed in at least thirteen different places scattered thoughout the two volumes, one has to concentrate hard to gain any overall picture of their function. With some larger topics, such as the effect of ageing or pregnancy on the skin, the problem is even more marked, with snippets of information scattered at innumerable places throughout the book.

Thus although this is certainly an impressive effort in terms of the breadth and depth of knowledge displayed, one emerges from a prolonged intellectual canter through its pages feeling overawed, but not necessarily much better informed. I must confess that I have the same problem with the British Museum, another magnificent thesaurus which leaves me similarly jaded. There are some notable exceptions to this criticism, and Wright's elegant and lucid review of epidermal cell kinetics, for example, shows how even the most complex subject can be made interesting and comprehensible to the non-specialist.

However, this book is not designed to be read from cover to cover, so I decided to test its worth by looking up ten topics chosen at random. The *Langerhans cell* received satisfactory coverage, with a chapter of its own, and six references dated as recently as 1981. *Chalones* were adequately discussed, though one had to look in five different places in the book. *Odland bodies* were not in the index, and membrane coating granules were mentioned only very briefly in relation to nail formation. Their role in keratinization of the hair follicle was not mentioned. I searched the index in vain for *wrinkles*, *lentigines* and *freckles* (and ephelides). I next needed to check the effect of *brown fat* on skin temperature. Brown fat was not indexed, and even white fat was dismissed by a single brief paragraph on its embryology. *Menopausal flushing* was given an index reference to page 1126, which deals with the carcinogenic effect of phorbol esters, but I eventually found an account of menopausal flushing seven chapters later. This discussed the effect of alcohol in menopausal flushing in some detail, but the role of oestrogens was not discussed, and the important association with pulsatile release of luteinizing hormone was not mentioned. *Placebo responses* were not in the index. My final test topic was the possible role of *vitamin A* in preventing skin cancer. The work on carcinogenesis in vitamin A deficient rats was not mentioned, but there was a brief account of the effects of retinoids in preventing cancer (with three references dated 1970, 1977 and 1979).

I awarded a score of 4 out of 10, and my admiration for Rothman increased enormously. My test topics admittedly had a clinical bias, and readers with a greater interest in Bz-arg-2-N Nap hydrolysing proteinase, or the levels of citrulline in the bovine snout epidermis might well have awarded a much higher score in a similar test.

The book is sumptuously produced with many fine black-and-white photographs, but it would be helpful to have the index in both volumes. There are hardly any printing errors, and even the table headed 'Changes in hare colour' referred to the effects of photoperiodism on the pelt of the Scottish mountain hare.

Despite my reservations, the book probably does achieve its stated aim of providing an authoritative and (reasonably) comprehensive review of the structure, physiology and biochemistry of the skin, and at present it has no rivals. I feel sure that the second edition, which will undoubtedly be required before long, will prove a worthy successor to 'Rothman'.

J.L.Burton

Pityriasis rubra pilaris—a summary of the literature from 1835–1982. W.Kuster (1983) Bibliotheca genetica medica, Vol 17. Pp. 86, Price DM 14.00.

Available from Palm & Enke, P.O.Box 41, D-8520 Erlangen, West Germany. This book is an alphabetical listing of over 1,000 references on pityriasis rubra pilaris, culled from the international literature.

Histopathology of the Skin. W.E.LEVER AND G.SCHAUMBURG-LEVER (1983) (6th Edition) Philadelphia: Lippincott. Pp. 848. Price £80.00.

One of the problems with new editions of established works is that they all too often offer little that is really new, merely representing regurgitations of previous editions with a few new references slotted into selected chapters. It is also rare to find in the sixth edition of a textbook the freshness of approach that may have been present in the original. Walter Lever is to be congratulated therefore on the new *Histopathology of the Skin*. This work has undergone one of the most major revisions in its history with a new printed format and layout, and extensive changes and additions to the individual entries.

The new volume has become larger, both in the page size itself and in the total number of pages. New type-faces have been used for headings and sub-headings and the technical quality of the photomicrographs has been improved. It is also pleasing to see that the majority of photomicrographs are placed close to the text which refers to them. Too often with illustrated textbooks one is forever turning pages to find a particular illustration or figure which is printed some way away from the written reference.

The great strength in this work, however, lies in the amount of new material. A total of one hundred and four conditions are discussed that were absent in the fifth edition of *Histopathology of the Skin*. These are listed for easy reference at the front section of the book under the headings 'new' diseases and 'newly included' diseases. In addition many of the other sections have been extensively rewritten to take account of recent advances in knowledge. The chapter on melanocytic naevi and malignant melanoma, for instance, addresses the problems of multiple primary melanomas, melanoma in children and the dysplastic naevus syndrome, as well as including a full discussion of histological prognostic factors in malignant melanoma.

Other features of this edition that have been usefully expanded and updated are the sections on histogenesis, where there is appropriate recognition and interpretation of research advances in electron microscopy, immunology, histochemistry and cell biology and the references at the end of each chapter. Many references in the old edition have been deleted and new references, several from as recently as 1982, have been included.

The amount of information in this new edition of 'Lever' is considerable and it is surely destined once again to become a best-seller not only to pathologists but also to discerning clinical dermatologists.

NEIL SMITH

Recent Advances in Dermatology No. 6. A.J.ROOK AND H.I.MAIBACH (1983) Edinburgh: Churchill Livingstone. Pp. 282. Price £22.00.

The 'Recent Advances in Dermatology' series has been a great success and No. 6 is as good as its predecessors. As usual it consists of a series of review articles. Ideally such a book should cover topics not easily found elsewhere. Chapters under this heading are 'Dermatology of the rickettsioses', 'Susceptibility to skin infection', 'The toxicology of hair dyes', and 'Flushing reactions'. All are fascinating chapters. All the other chapters are very well written and of interest, although the contents are fairly easily available elsewhere. The reviewer particularly enjoyed 'Human papovaviruses', 'Atopic dermatitis', 'Psychiatry and skin disease', and 'Synthetic retinoids'. The chapter on antihistamines does not mention the new non-sedating antihistamines, but this is the result of the inevitable delay when review articles are published in book form.

In conclusion, this is an excellent series of review chapters and is of interest and value to all clinical dermatologists. It is recommended reading.

A.P.WARIN

British Journal of Dermatology (1984) **110,** 510.

News and Notices

Skin Pathology Course, Toronto, July 1984

The Dermatopathology Foundation's 12th Annual Course in Gross and Microscopic Pathology of the Skin will be held on 9–13 July 1984 at the Delta Chelsea Inn, Toronto, Ontario, Canada. For further details write to: Dr M.R.Okun, Dermatopathology Foundation, P.O. Box 377, Canton, MA 02021, U.S.A.

Symposium on the Treatment of Psoriasis, Israel, October 1984

The Third International Symposium on the Treatment of Psoriasis and Psoriatic Arthropathy will be held in Herzlia, Israel from 28 October to 2 November 1984. For further details please contact: Nahum D. Yahalom, Organiser, Health Vacation Centre, P.O. Box 99, Ramat Hasharon 47100, Israel.

Hellenic Dermatological Congress, Athens, April 1984

The 4th Hellenic Dermatological Congress will be held in Athens, Greece on 12–14 April 1984. The main topics covered will be Clinical Dermatology, Cosmetic Dermatology and Sexually Transmitted Diseases. For further details please write to: Professor J.Stratigos, Athens University, Dermatology Clinic, 'Andreas Syngros' Hospital, I. Dragumi 5, Athens 508, Greece.

Practical Skin Pathology Course, Liberty, U.S.A., August 1984

A course on practical skin pathology will be given at Grossinger's, Liberty, New York on 19–24 August 1984. This is a comprehensive and practical update of dermatopathology for pathologists and dermatologists, sponsored by the Department of Dermatology, New York Medical College-Metropolitan Hospital Center. Microscopes and syllabus material will be provided. There will be 30 formal lectures and 8 supervised microscope sessions (32 hours) of 400 unknown workshop slides. For further information, please write to Martin H. Brownstein, M.D., Skin Pathology Course, 2 Jordan Drive, Great Neck, New York 11021, U.S.A., or phone (516) 829-8578.

Royal College of Physicians and Surgeons of Glasgow

Dr W.S.Douglas was elected to Fellowship of the Royal College of Physicians and Surgeons of Glasgow in October, 1983.

Correction

D.M.WILLIAMSON and RACHEL GREENWOOD, Multiple pyogenic granulomata occurring during etretinate therapy. *British Journal of Dermatology* 1983, **109,** 615–619. We apologise for the fact that a sentence was omitted from the above letter. 'He was then started on etretinate 25 mg t.d.s.' should have been inserted in paragraph three, after the sentence 'Endoscopy suggested Crohn's disease and the methotrexate therapy was stopped'.

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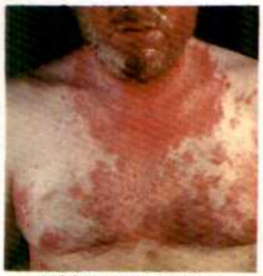
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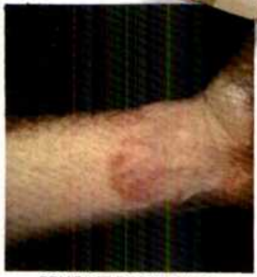
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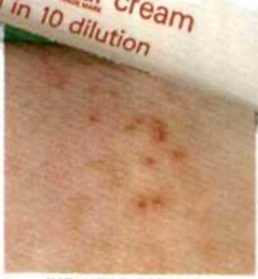
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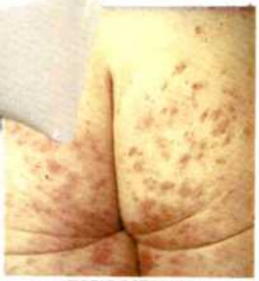
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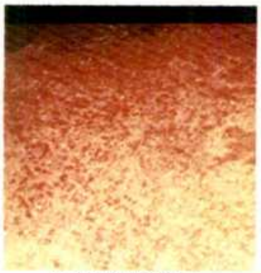
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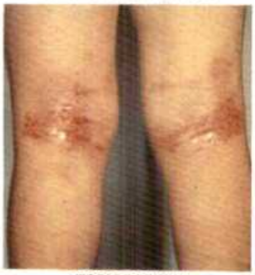
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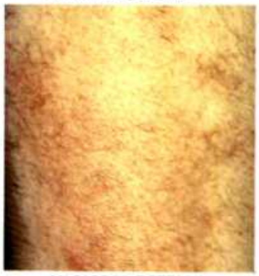
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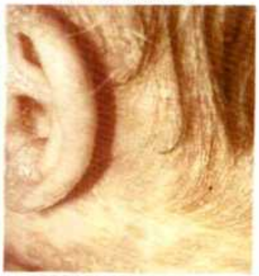
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Pocket Atlas of Dermatology

By Gerd Klaus Steigleder,
Director, Universitäts-Hautklinik, Köln

Howard I. Maibach
Dept. of Dermatology, University of California
School of Medicine, San Francisco, California/USA

1984. 280 pages, 292 colored figures,
<Thieme Flexibook> approx. $ 14.00/DM 34,-

A brief and concise survey of the most important dermatologic and venereologic disease patterns, explained by means of tables and, above all, color illustrations. The illustrations have been selected mainly under the aspect of differential diagnosis in general practice. The text is largely oriented on the basis of the index. The atlas is supplemented by the pocketbook „Dermatology".

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	**g**
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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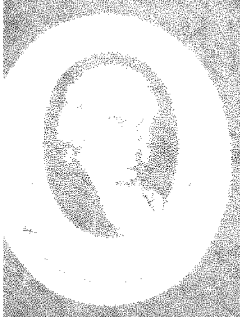

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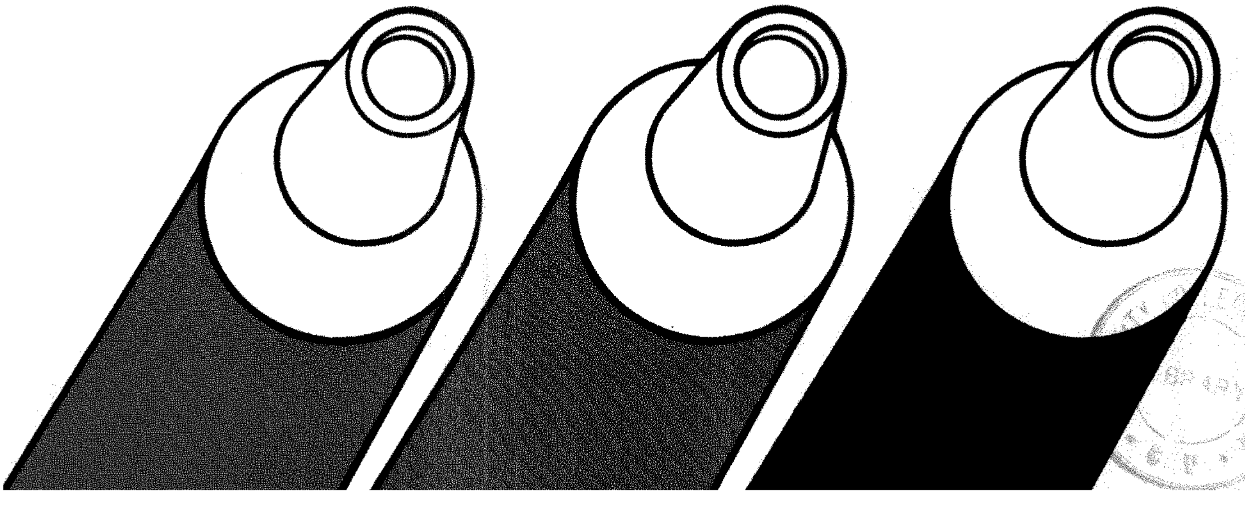

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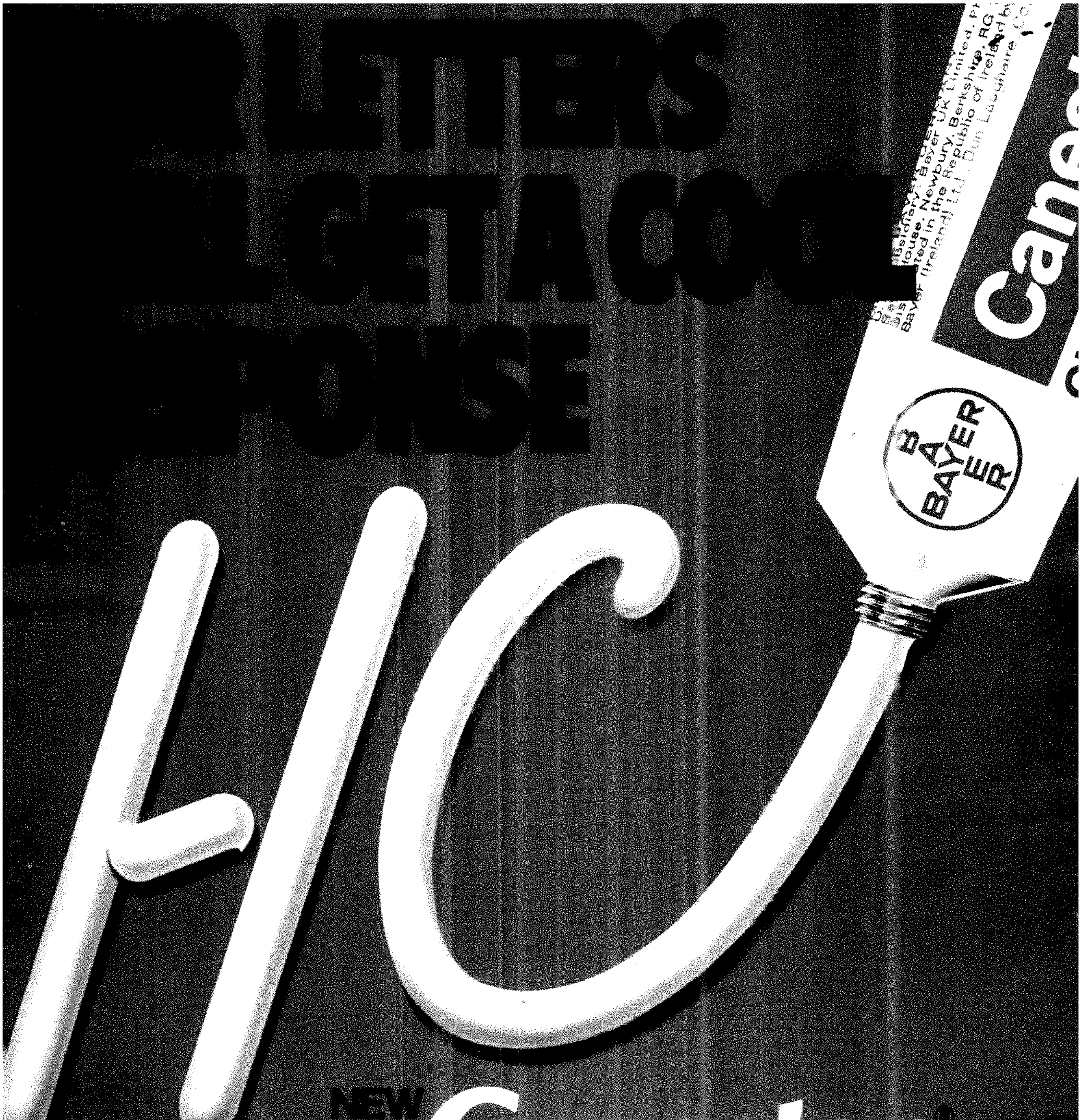

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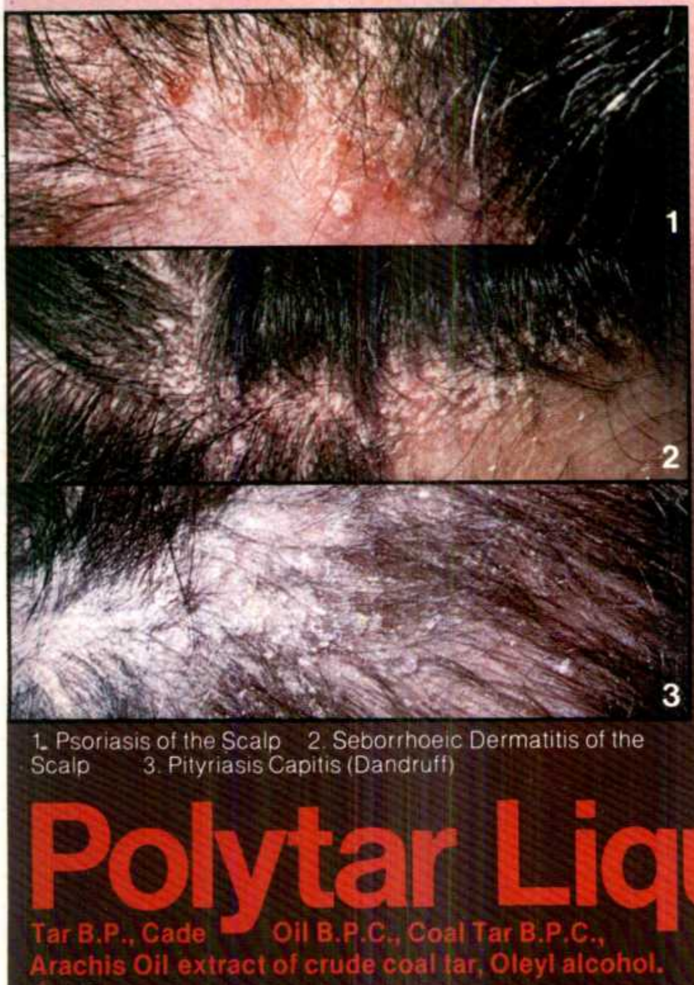

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Oleyl alcohol	1.00%

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Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

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Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

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Legal category: P
Package quantities: Bottles containing 18 ml with brush fitted in cap.
Further information: Nil.
Product licence number: 0118/5010.
Basic NHS price: £0.57 per 18 ml bottle.

Reference

1. Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; 214: 657-63.

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Fry L. Update: Mar 1978 681-69

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rescribing information

osage and administration

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ide effects

ith all topical corticosteroids local atrophic changes may ossibly occur following prolonged and intensive treatment. lso, prolonged use of large amounts or treatment of xtensive areas may produce the features of hypercorticism. his is more likely to occur in infants and children, and with cclusion. In infants, the napkin may act as an occlusive ressing.

rovided the weekly dosage of Dermovate is less than 50 rams in adults, any pituitary-adrenal suppression is likely to be ansient with a rapid return to normal values once the short ourse of steroid therapy has ceased. The same applies to hildren given proportionate dosage.

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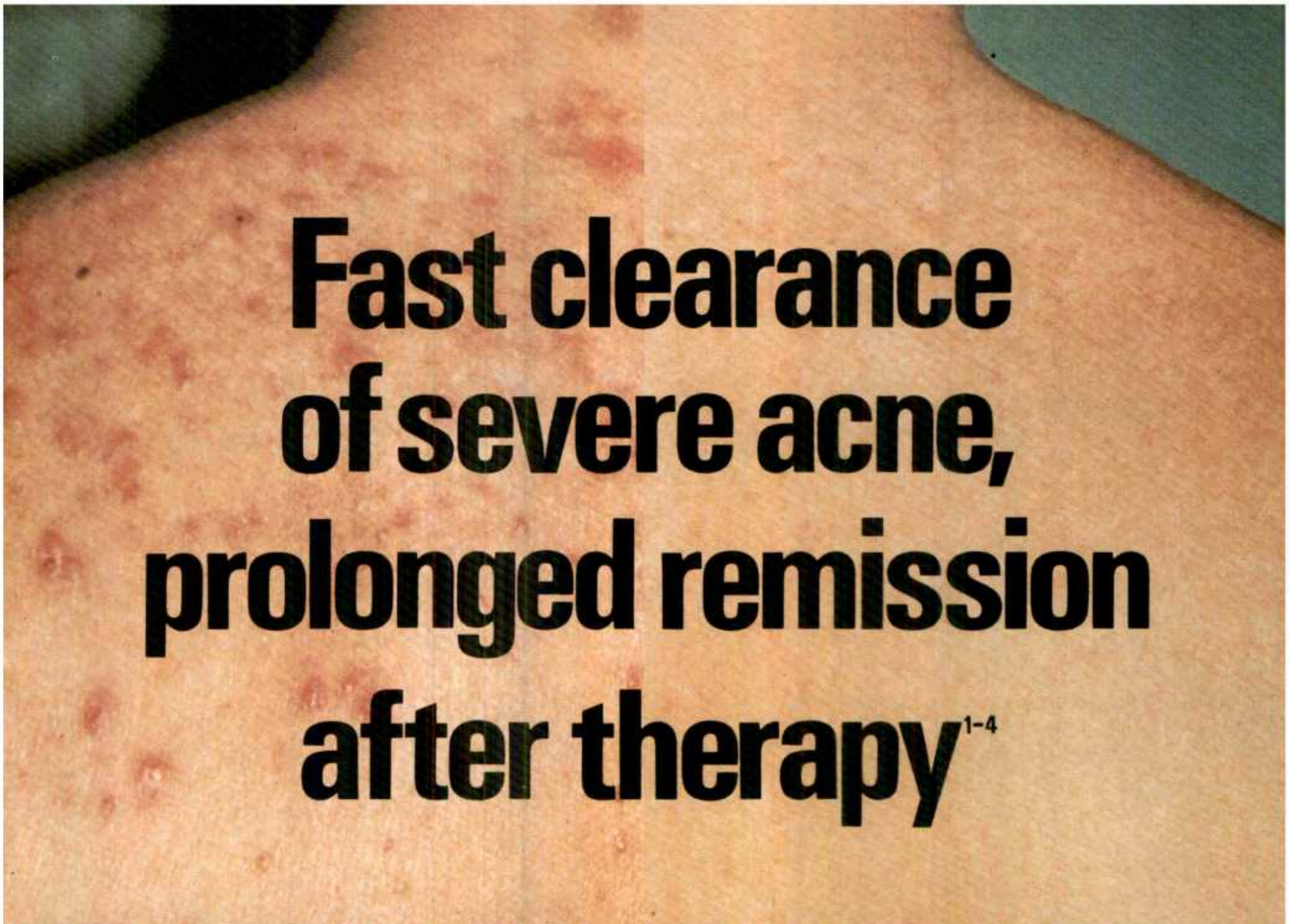

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Contra-indications Pregnancy (Roaccutane is teratogenic—see 'Precautions') and breast feeding. Hepatic or renal impairment.

Precautions The following precautions must be observed in women of childbearing potential.

1. Patients should be given detailed explanation of the risks.
2. Pregnancy should be excluded before therapy is instituted.
3. Effective contraception must be ensured for one month before treatment, during treatment, and for one month after treatment ends.

Use of patient information leaflets is strongly recommended. Monitor liver function and serum lipids (fasting value) regularly.

Side-effects Mainly mucosal dryness and fissures, facial dermatitis, desquamation, epidermal fragility, conjunctivitis, arthralgia/myalgia, increased hair loss (uncommon and reversible), possible elevation of liver enzymes and serum triglycerides. Benign intracranial hypertension has been reported, particularly in association with concomitant antibiotics.

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Presentations and Basic NHS Cost

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References

1. In: "Retinoids: Advances in Basic Research and Therapy", Eds. Orfanos, C.E. *et al.*, Springer-Verlag, Berlin, 1981, p.219.
2. Brit.J.Derm., 1983, *108*, 333.
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4. J.Amer.Acad.Derm., 1982, *6*, 766.
5. J.Amer.Acad.Derm., 1983, *9*, 629.

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Abstract

Arbeitsgemeinschaft Dermatologischer Forschung (ADF) in Cooperation with „Deutsche Dermatologische Gesellschaft" X Annual Meeting November 19–21, 1982, Muenster, FRG 263

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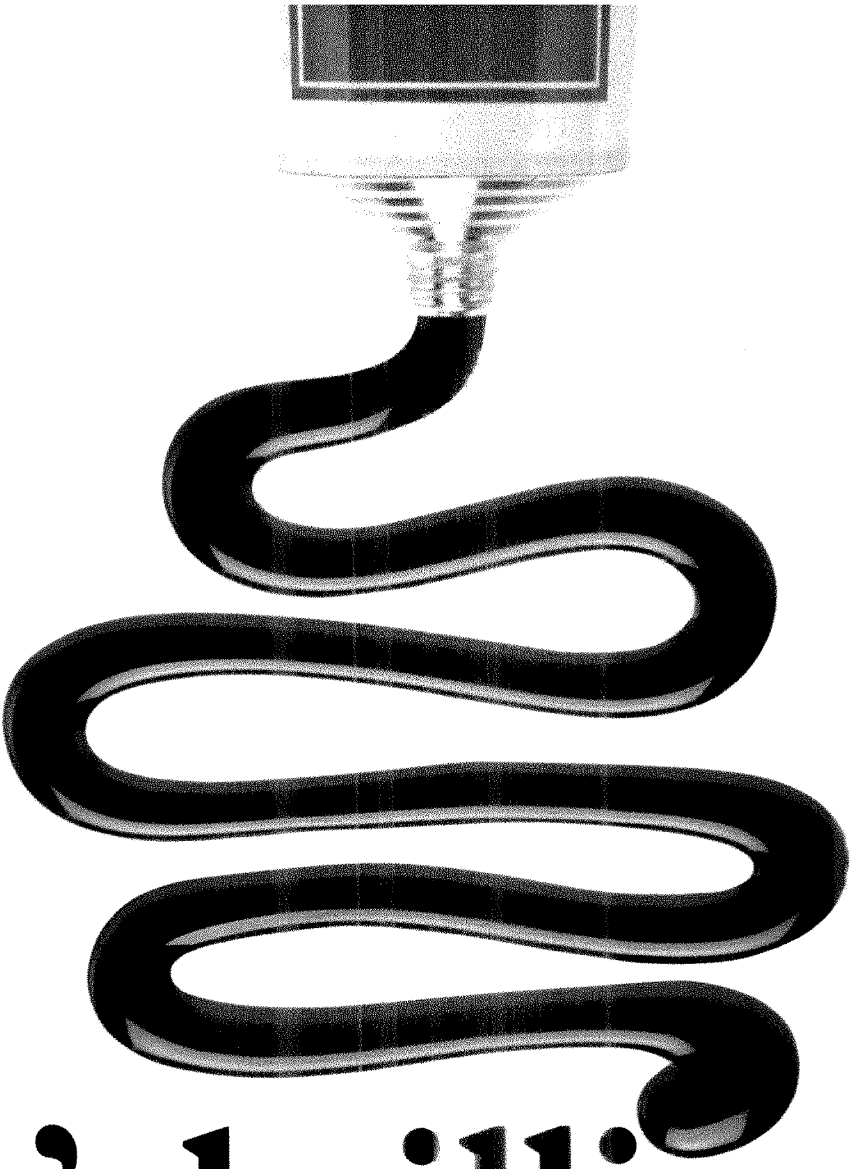

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Clinical and Laboratory Investigations

The diagnosis of cutaneous T-cell lymphoma by morphometric evaluation of the cellular infiltrate, using semithin sections

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SUMMARY

In order to improve the cytological criteria for the diagnosis of cutaneous T-cell lymphomas (CTCL), a number of morphometric assessments in semithin sections have been performed on the dermal infiltrates of twenty-one cases of overt CTCL (group I) and twenty-two cases of well-defined benign dermatoses (group II). In each biopsy, an average of 250 cells were measured for perimeter (P), surface area (S), 'nuclear shape index' (NSI), mean surface and mean NSI. We also determined the percentage of typical 'highly cerebriform lymphocytes' (HCL).

Cells of group I patients were found to have mean S and mean NSI that differed significantly from those of group II. HCL were found to have a mean NSI value of less than 0·40. The diagnostic value of these measurements was confirmed in the group I patients. Nineteen of them had a mean NSI value of less than 0·61, together with a mean S greater than 14 sq. μm. None of the twenty-two patients of group II had such values.

The discriminating power of these criteria was then tested retrospectively on another group of initially controversial patients who presented with suspected CTCL (group III patients). Nine have since evolved into overt CTCL (group III M) while the other ten have remained benign (group III B). All group III B patients and five patients of group III M were correctly assigned using the above morphometric criteria.

This method could improve the diagnosis of the early stage of cutaneous T-cell lymphoma.

In cutaneous T-cell lymphomas of the mycosis fungoides (Sézary) type the malignant cells display remarkably convoluted nuclei on electron microscopy (EM). Light microscopy is less valuable for detecting such nuclear abnormalities because of the thickness of conventional tissue sections (Lefeber *et al.*, 1981). Another limitation stems from the fact that similar if not identical

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cerebriform cells can be found in a number of benign lesions (Flaxman, Zelazny & Van Scott, 1971; Ruttner & Pedio, 1976). In some chronic dermatoses both the clinical and histological features may be equivocal, making the diagnosis of early cutaneous lymphoma doubtful.

Thus there is a need for a more stringent histological definition of the malignant T-cell infiltrate. Guccion *et al.* (1979) and Rosas-Uribe *et al.* (1974) proposed that clustering of the cerebriform cells would provide a more precise diagnostic approach, but Van der Loo *et al.* (1980) and McNutt, Heilbron & Crain (1981) showed that semi-quantitative appreciation of nuclear indentations in suspected cells could provide a more discriminating measurement. The so-called 'nuclear contour index' (NCI) is essentially based on a semi-automated analysis of electron micrographs.

In the present study, a number of morphometric evaluations of lymphoid cell nuclei have been performed under light microscopy. This was made possible by using semithin sections of epoxy-resin-embedded specimens, a technique already used by some pathologists for better definition of the cells (Bryon, 1976).

METHODS

Patients

Group I consisted of twenty-one patients (eleven males and ten females) with clinically and

TABLE 1. Patients with definite cutaneous T-cell lymphoma (group I)

Patient No.	Diagnosis	Sézary cells (%) in blood
7	MF I B*	< 5
67	MF I B	< 5
28	MF I B	< 5
23	MF I B	< 5
25	MF I C	< 5
56	MF I C	< 5
4	MF II B	< 5
12	MF II B	< 5
5	MF II B	< 5
29	MF II C	< 5
51	MF II C	< 5
1	MF III C	< 5
38	MF II C	< 5
58	MF II D	17
27	MF II D	22
14	MF IV C	25
8	MF III C	
3	MF III C	30
26	MF III D	8
30	MF IV D	
37	MF IV C	

* MF = Mycosis Fungoides; A = eczematous eruption; B = infiltrating plaques; C = skin tumours; D = erythroderma.

histologically diagnosed CTCL (Table 1). The patients were staged according to the classification of Fuks, Bagshaw & Farber (1973).

Group II consisted of twenty-two patients with various chronic benign dermatoses: four lymphocytic infiltration of Jessner, two prurigo, two discoid lupus, five chronic eczema, four lichen planus, five parapsoriasis (benign type).

Group III was a 'controversial' group of nineteen patients presenting with chronic and/or relapsing dermatoses, all with some features suggestive of CTCL (Table 2). The histological suspicion was raised by the finding of clustered cerebriform cells in the dermal infiltrate. These

TABLE 2 Clinical details of nineteen initially controversial patients (group III) Abbreviations: as for Table 1

Patient No	Initial diagnosis	Follow-up after initial biopsy (years)	Final diagnosis
64	Light-provoked eczema? Actinic reticuloid? CTCL?	2	Photosensitive eczema
52	Eczematous chronic dermatitis	2	Gold salt eruption Now in remission
42	Chronic psoriasiform dermatitis	4	Diagnosis confirmed. Improved by PUVA
13	Itching relapsing eczematous plaques	3	Unlabelled dermatitis still present
40	Prereticulotic parapsoriasis	5	Diagnosis unchanged. Remission with mechlorethamine
69	Poikiloderma atrophicans vasculare (PAV)	4	PAV confirmed Remission with mechlorethamine
39	Poikiloderma atrophicans vasculare (PAV)	4	PAV confirmed Incomplete remission with mechlorethamine
68	Atopic dermatitis with generalized skin thickening and peripheral nodes	4	Atopic dermatitis
6	Atopic dermatitis with lichenification	4	Atopic dermatitis
50	Atopic dermatitis with lichenification	3	Atopic dermatitis
17	Itching, relapsing, psoriasis-like eruption	3	MF I B
24	Erythroderma	5	MF II B
53	Erythroderma	5	MF II D
11	Mycosis fungoides in clinical remission	5	MF IV C
44	Erythroderma	4	MF II DC (16%)
18	Eczematous chronic dermatitis	6	MF I B
60	Erythroderma	0·5	MF III D
61	Mycosis fungoides in clinical remission	4	MF IV D
16	Erythematous patches and plaques	4·5	MF I B

were mostly of the small size type. Only a few infiltrates displayed the typically large Sézary cells with markedly convoluted nuclei which predominated in group I patients.

Each case in group III was retrospectively included only if after surveillance of at least 2 years the initially controversial dermatosis could be assigned with certainty to either a benign (III B, ten patients) or a malignant (III M, nine patients) outcome.

Morphometric analysis

The nuclei of the lymphoid cells were analysed with a semi-automated system of picture analysis (ASM-Leitz device; Leitz, West Germany). This device comprises a digital table on which the operator outlines the periphery of the nucleus using an electronic pencil. The table is connected to both the microscope and microcomputer. The microscope is a tungsten-lighted Leitz Dialux 20 equipped with a transparent chamber and with a Weibel micro-network incorporated within one of the ocular lens and with an ocular micrometering system.

The perimeter and two-dimensional surface area of each nucleus was directly evaluated by outlining the contour. Perimeters were measured in microns and surfaces in sq. μm. They were computed after determination of a magnification factor obtained in reference to an 'artificial lymphocyte' printed on a microscopic glass slide (Larc Analyser, STD test slide Rev A 284, Corning).

From these two measurements a 'nuclear shape index' (NSI) was computed according to the formula:

$$\mathrm{NSI} = 4\pi \frac{S}{P^2}.$$

The nuclear shape index is related to the degree of nuclear indentation independent of the size of the cell. An index of 1 denotes a circular nucleus. Lower indices express a larger perimeter relative to a given surface and thus a more convoluted nucleus.

NSI can be linked to 'nuclear contour index' (NCI) according to the following transformation: NSI = 4π NCI (accordingly, 3·55 is the NCI value for a circular nucleus).

Drawing procedure

Prior to collecting any data, the operator sharply focussed the microscope on the cell to be drawn and concurrently performed a fine tuning of the ocular lens of the Weibel reticule and of the electronic pencil.

The focus was not altered during any of the experimental measurements. The stability was checked on the artificial lymphocyte, on which surface and perimeter were regularly determined several times, five times each, before and after examination of tissue sections.

Examination of tissue sections was performed in the following way. The borders of the sections where cells were likely to be damaged were not considered. The immersion objective lens (× 100) was pointed to the basal layer of the epidermis, starting from one side of the section. After having screened the entire field, the adjacent deeper field was examined and so forth down, then back up to the first field, on to the next contiguous superficial one and then down again, field by field.

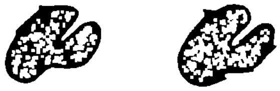
Within a given field, the cells were depicted from left to right and from top to bottom following the linings drawn on the Weibel reticule. The nuclear contour of each lymphoid cell within the reticule was drawn in a clockwise way.

Only cells clearly assignable to the lymphoid series (see below) and with a sharply defined nuclear contour were selected. It is important to distinguish the lymphoid from the histiocytic cells since some histiocytes may present with profound nuclear indentations. However, the nuclei of histiocytes appear lighter, they often have a central nucleolus and do not show a clear peripheral density of chromatin. They are easily recognized when phagocytic material is present in their cytoplasm.

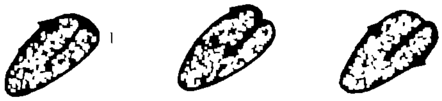
When specimens were quite broad and/or densely infiltrated, horizontally adjacent fields parallel to the epidermis were sampled.

The nuclear indentation of cerebriform cells is depicted in Fig. 1. With 'chicken foot' indentations the starting point of the electronic pensil was chosen as the minimal distance between 'chicken foot' and nuclear membrane (Fig. 1). Such artefactual tricks were necessitated by the inalterable focusing stand, as opposed to the natural observation procedures where constant refocusing is required for a sharp appreciation of deep indentations in a given nucleus.

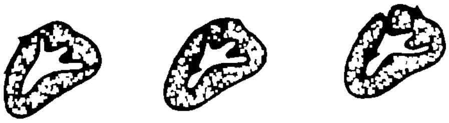
The number of the nuclei thereby evaluated on each section varied from 117 to 250, depending on the actual number of screenable cells in the infiltrate.



(a) Evident indentation



(b) Indentation presenting as a single line



(c) 'Chicken foot' indentations

FIGURE 1. The scheme used to draw the nuclear indentations.

RESULTS

Reproducibility

Repeated measurements of the same cells by the same examiner are shown in Table 3. Some examples of sketches are presented in Fig. 2, together with the NSI and surface values of the corresponding lymphocytic cells. In this study, a 'highly cerebriform' cell produces a NSI of 0·40. The percentage of such highly cerebriform lymphocytes (HCL) can be calculated.

Morphometric data within group I as compared with group II

For each patient, the values obtained from each cell were averaged, yielding a mean NSI and a mean surface area. The percentage HCL was also obtained. Figure 3 shows the distribution of mean NSI and mean surface area for each patient of each group.

Within each group the means for mean nuclear shape index, surface and percentage of HCL were calculated. Although there was some overlap the differences between the means of the groups were statistically significant (Table 4). From those data, the value which best

TABLE 3. Reproducibility of surface and nuclear shape index (NSI) measurements

Cell number	Surface*	NSI*
1	11·29	0·15
	13·37	0·15
	13·34	0·16
	13·30	0·17
	13·27	0·16
2	17·17	0·28
	15·35	0·28
	16·52	0·27
	16·22	0·26
	16·22	0·27
3	12·37	0·25
	12·52	0·26
	13·32	0·28
	13·25	0·27
	13.28	0·26
4	11·48	0·37
	11·46	0·41
	11·72	0·40
	12·04	0·40
	11·95	0·40

* Each cell was measured five times by the same person.

discriminated between group I and group II was less than 0·61 for NSI and greater than 0·165 for percentage of HCL. Both values were equally sensitive for the diagnosis of CTCL, though percentage of HCL was more specific. In one case from group I neither values were obtained: this patient presented with a tumour form of mycosis undergoing an immunoblastic transformation (Table 5).

Among the twenty-two cases of group II only two had a percentage of HCL greater than 0·165 (case 45: lichen planus; case 63: digitate parapsoriasis). Those two patients also had a NSI value less than 0·61 (Table 5). The other patients with digitate parapsoriasis (cases 54, 62) gave a mean NSI value less than 0·61.

However, by resorting to both mean NSI and mean S (Fig. 3), group I and group II were efficiently separated. Indeed, a mean NSI less than 0·61 together with a mean S greater than 14 sq. μm provided both specificity (no false positive) and good sensitivity (Table 5). However with these criteria two patients from group I were excluded. One had an immunoblastic transformation as already mentioned (case 37), the other one had mycosis presenting as a single infiltrated plaque (case 25).

Morphometric data within group III and comparison between groups III M and III B

The mean differed significantly between the two groups (Table 6). The mean percentage of HCL was higher in group III M and the mean NSI was lower in group III M without reaching statistical significance.

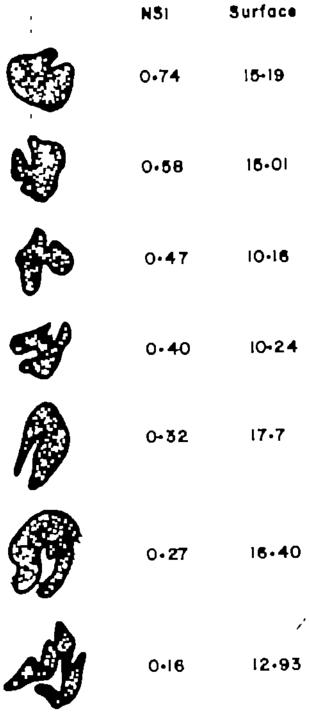


FIGURE 2. Some examples of nuclear shape index (NSI) and surface (S) values together with outlines of the corresponding lymphocyte nuclei.

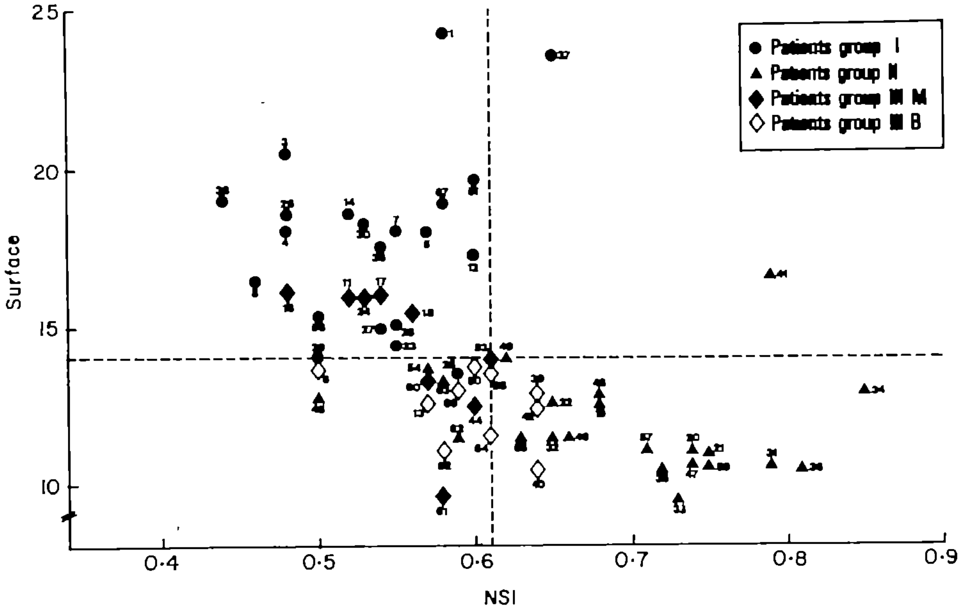


FIGURE 3. Distribution of means of surface and NSI for each case of groups I, II and III. Dotted lines (horizontal at 14 sq. μm, vertical at 0 61) have been added empirically for a better delineation of groups I and II.

TABLE 4. Comparison of group I and group II using patients' means for nuclear shape index (NSI), percentage of highly cerebriform lymphocytes (HCL) and surface (S)

	Mean NSI	% HCL	Mean S
Group I (*n* = 21)	0·54 ± 0·01*	25·4 ± 1·8	17·8 ± 0·61
(Range)	(0·44–0·65)	(6·8–43·2)	(13·5–24·4)
Group II (*n* = 22)	0·69 ± 0·02	7·33 ± 1·4	11·88 ± 0·33
(Range)	(0·50–0·85)	(0·8–26·4)	(9·49–16·51)
P value (Mann–Whitney)	$< 10^{-5}$	$< 10^{-6}$	$< 10^{-7}$

* Mean ± standard error of mean.

TABLE 5. Morphometric diagnostic criteria for cutaneous T-cell lymphoma (group I) as compared to benign conditions (group II)

Criteria of CTCL	% HCL > 16·5	Mean NSI < 0·61	Mean NSI < 0·61 and Mean S > 14 sq. μm
Sensibility	95%	95%	90%
Group I (*n* = 21)			
(False negative)	(37)*	(37)	(37,25)
Specificity	90%	82%	100%
Group II (*n* = 22)			
(False positive)	(45,53)*	(45,65,54,62)*	

* Patient number.

TABLE 6. Comparison of morphometric measurements in the controversial group (III M: malignant outcome) (III B: benign evolution)

	Mean NSI	% HCL	Mean S
Group III M (*n* = 9)	0·55 ± 0·014	20·3 ± 3·12*	14·28 ± 0·75
(Range)	(0·48–0·61)	(10·8–24·4)	(9·5–16·2)
Group III B (*n* = 10)	0·60 ± 0·013	15·0 ± 2·21	12·4 ± 0·35
(Range)	(0·57–0·64)	(7·6–32·4)	(11·1–13·8)
P value (Mann–Whitney)	> 0·05 (NS)	> 0·05 (NS)	< 0·05

* Mean ± standard error of mean.

TABLE 7. Sensitivity and specificity of morphometric criteria in controversial group (III M: malignant outcome) (III B: benign evolution)

Criteria of CTCL	% HCL > 16·5	Mean NSI < 0·61	Mean NSI < 0·61 and Mean S > 14 sq. μm
Sensibility Group III M ($n=9$)	44%	89%	56%
(False negative)	(61,18,53,44,60)*	(53)	(44,53,60,61)
Specificity (Group III B ($n=10$)	80%	50%	100%
(False positive)	(6,13)	(6,13,50,52,69)	

* Patient number.

The discriminative values determined for group I and group II patients were applied to group III (Table 7). Mean NSI less than 0·61 appears as non-specific although highly sensitive, with only one case from group III M with a value of 0·61 (case 53).

However, the specificity is satisfactory when the two criteria are combined (mean NSI less than 0·61 and mean S greater than 14 sq. μm). Indeed all cases from group III B were excluded according to these criteria as compared to only four of nine with a malignant outcome (III M). Of these four, three (cases 44, 60, 61) had a mean NSI less than 0·61 but a mean S less than or equal to 14 sq. μm. Thus they appear on Figure 3 in the lower left quadrant, next to one patient from group I (case 25), four patients from group II (45, 63, 54, 62) and five cases from group III B (6, 13, 50, 52, 69).

DISCUSSION

In the present work, morphometric data provide the basis for objective quantitative and reproducible characterization of typical cells of cutaneous T-cell lymphomas. These measurements were performed on cutaneous Epon-embedded specimens allowing semithin sections and trichromic staining. The whole operation can be carried out under light microscopy.

The convoluted pattern of lymphocyte nuclei as assessed either by mean NSI or by percentage of HCL alone does not allow a satisfactory discrimination between malignant and benign groups, but a better separation of these groups can be obtained by combining both measurements (mean NSI and mean S).

We have applied this same method to a controversial group of patients who could not be classified either by clinical or histological non-morphometric criteria. In this way, we identified ten out of ten patients who were eventually confirmed to have benign dermatosis, and five out of nine patients who eventually developed overt malignancy.

Thus our method seems to provide a better discriminative tool than subjective evaluation, even on semithin sections where cerebriform cells can be easily seen. Infiltrates found in the early phases of MF are often scanty and poor in highly cerebriform cells, and the pathologist finds it difficult to memorize differences in the shape of nuclei which do not appear very often.

Four out of nine controversial conditions which eventually became malignant could not be predicted as such on the basis of our criteria. It may have been that the sensitivity of our method

Library

was insufficient, possibly hampered by a methodological bias, deliberately excluding 'exocytosed' cells.

On semithin sections, lymphoid cells in the epidermis are usually compressed between keratinocytes. Such cells are very rarely found in full section, allowing their measurement, and even though exocytosis of some degree is a helpful feature in diagnosing cutaneous T-cell lymphomas, it remains but one factor contributing to such a diagnosis.

One must also consider the possibility that all the cells with a convoluted nuclear membrane are not in fact lymphocytes. In fact, some of the cells with sinuate irregular nuclear shape belong to the histiocyte series (i.e. macrophages or Langerhans cells). This has been confirmed by examination of some of our epoxy-resin-embedded specimens under electron microscopy. These observations had been recently confirmed by the avidin–biotin complex technique (Hsu, Raine & Fanger, 1981) which demonstrated that OKT6-positive cells were present in the papillary dermis, scattered among the lymphoid cells. The fact that we did not consider those cells not clearly assignable to the lymphoid series, could account for our underestimation of the infiltrates.

An alternative explanation for the relative lack of sensitivity of our evaluation of 'premalignant' lesions can also be proposed. The so-called premycotic states include various entities which share a common factor of chronic cellular stimulation, whether viral or other unknown stimulating agents are involved (MacKie, 1981). Such chronic inflammatory states would favour the occurrence of a second transformant (i.e. oncogenic) event (Poiesz *et al.*, 1980). These lesions could facilitate the development of cutaneous T-cell lymphoma. Premycotic states could therefore be more difficult to recognize on a mere morphological basis.

Using other semi-quantitative criteria, Van der Loo *et al.* (1980) and McNutt *et al.* (1981) have established highly discriminative indices for recognizing malignant and non-malignant cells in the skin. The level of sensitivity which they attained was comparable to ours, but they based their evaluation on electron rather than light micrographs, thus increasing the cost of their investigations.

In conclusion, semithin sections combined with morphometric analysis appear to provide an efficient tool for investigation of the cellular infiltrates of cutaneous lymphomas.

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Lymphocytic infiltration of the skin (Jessner): a T-cell lymphoproliferative disease

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SUMMARY

Recent studies have suggested that Jessner's lymphocytic infiltration of the skin and lymphocytoma cutis both represent pseudo-B-cell lymphomas. We have now done immunohistochemical studies on frozen skin sections of six patients with lymphocytic infiltration of the skin. In all cases a predominance of T lymphocytes was observed and B lymphocytes were sparse or completely absent. These results indicate that Jessner's lymphocytic infiltration of the skin is a T-cell lymphoproliferative disease which, unlike lymphocytoma cutis, cannot be regarded as a pseudo-B-cell lymphoma.

Lymphocytic infiltration of the skin (LIS), first described in 1953 by Jessner and Kanof, is a relatively uncommon disorder, characterized clinically by the presence of erythematous, peripherally enlarging papules or plaques, which may show central clearing. They are most commonly located on the face and upper back. Histologically the lesions show patchy, perivascular and sometimes periadnexal lymphocytic infiltrates. The clinical and histological similarities to discoid lupus erythematosus, the plaque stage of polymorphous light eruption, cutaneous pseudolymphomas such as lymphocytoma cutis (sarcoid of Spiegler–Fendt) and cutaneous localization of well-differentiated lymphocytic lymphomas, have resulted in controversy about the nosological position of this condition.

In 1969 Caro and Helwig grouped together a number of dermatoses with histological features simulating malignant lymphoma, including LIS, lymphocytoma cutis and insect bite reactions. For the whole group of diseases the term cutaneous lymphoid hyperplasia was proposed. For many years the relationship between LIS and lymphocytoma cutis has been a matter of considerable debate (Calnan, 1957; Wolf, 1957; Postma & Sluiter, 1958; Lange Wantzin *et al.*, 1982). Recent studies have suggested that both diseases represent pseudo-B-cell lymphomas (Burg *et al.*, 1982) but whereas the B-cell character of the dermal infiltrates in lymphocytoma

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cutis has been well established (Braun-Falco & Burg, 1975), immunohistochemical support for the supposed B-cell origin of the lymphoid cells in LIS is lacking.

In the present study we have performed immunohistochemical studies on frozen skin sections of six patients with LIS by means of a series of monoclonal antibodies. In all patients the dermal infiltrates were predominantly composed of T lymphocytes, indicating that LIS is a T-cell lymphoproliferative disease, and therefore not related to lymphocytoma cutis.

METHODS

Patients

Six patients (three males, three females) with lymphocytic infiltration of the skin (Jessner) were selected for this study. The age of onset of the skin lesions ranged between 25 and 46 years (mean: 36·6 years). The duration of the disease was more than 4 years in five of the six patients. Clinically all patients showed well-demarcated, infiltrated, non-scaling reddish plaques on either the face or upper back. Follicular hyperkeratosis or atrophy were not observed. In none of the patients was there an association between sun exposure and the occurrence of the skin lesions. Histologically all six cases showed well-demarcated perivascular infiltrates of varying density (Fig. 1). The infiltrates which tended to be more pronounced in the lower parts of the dermis, were predominantly composed of small lymphocytes. In addition scattered macrophages and occasional plasma cells were observed. The epidermis was unaffected in all cases. Immunofluorescence studies for the demonstration of immunoglobulins and complement at the dermal-epidermal junction were negative.

Immunohistochemical examinations

From all patients, unfixed frozen sections were investigated for reactivity with a series of monoclonal antibodies, including Leu-1, Leu-2a, Leu-3a, HLA-DR (Becton Dickinson, Facs. Systems, Sunnyvale, CA), OKT6, OKM1 (Ortho Pharmaceuticals Ltd, Raritan, NY), and TA-1 (kindly provided by Dr J.H.Kersey). The specificities of these antibodies are given in Table 1. Rabbit antisera against IgM and kappa and lambda light chains (Dakopatts, Denmark) were used for demonstration of B lymphocytes.

For *in situ* demonstration of antigens reactive with the aforementioned antibodies an indirect immunoperoxidase technique was used, as recently described (Willemze *et al.*, 1983).

RESULTS

In the dermal infiltrates of all six patients with LIS a predominance of T lymphocytes, as defined by a positive staining with the pan-T-cell antisera Leu-1 and TA-1, was observed (Fig. 2). The percentages of T cells ranged from 40 to 80% (Table 2). In all cases the numbers of Leu-3a$^+$ cells (helper/inducer T-cell phenotype) exceeded the numbers of Leu-2a$^+$ cells (cytotoxic/suppressor T-cell phenotype). The Leu-3a/Leu-2a ratios varied between 2:1 and 6:1 (Fig. 3).

Varying numbers of cells (15–30%) strongly reactive with anti-HLA-DR antiserum were found scattered between the lymphoid cells (Fig. 4). These cells, which often showed dendritic cytoplasmic extensions, probably represent macrophages. The large majority of the lymphoid cells appeared negative when stained with anti-HLA-DR antiserum.

Cells reactive with OKT6 antiserum were completely lacking in the dermal infiltrates of five patients with LIS. In one patient occasional OKT6$^+$ dendritic cells, presumably Langerhans

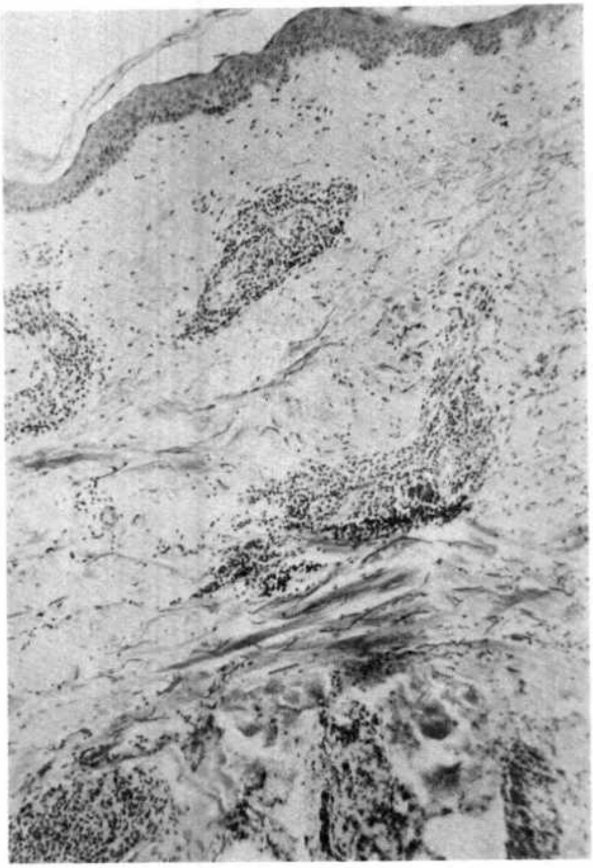

FIGURE 1. Lymphocytic infiltration of the skin. Well-demarcated predominantly lymphocytic perivascular infiltrates in the dermis. (H & E, × 64.)

TABLE 1. Specificity of monoclonal antibodies

Monoclonal antibody	Specificity
Leu-1	95% human thymocytes, >95% peripheral blood T cells, some SIg$^+$ CLL cells
Leu-2a	Cytotoxic/suppressor T-cell subset,
Leu-3a	Helper/inducer T-cell subset
TA-1	100% peripheral blood T cells, 70% thymocytes, >95% peripheral blood monocytes
HLA-DR	B lymphocytes, monocytes/macrophages, activated T cells
OKT6	70% thymocytes; Langerhans cells

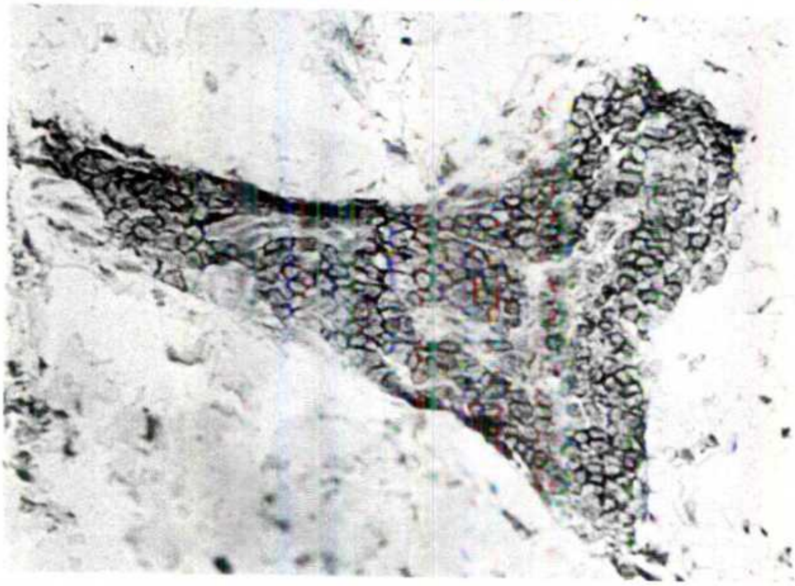

FIGURE 2. Lymphocytic infiltration of the skin. Perivascular infiltrate showing a predominance of Leu-1$^+$ T cells.

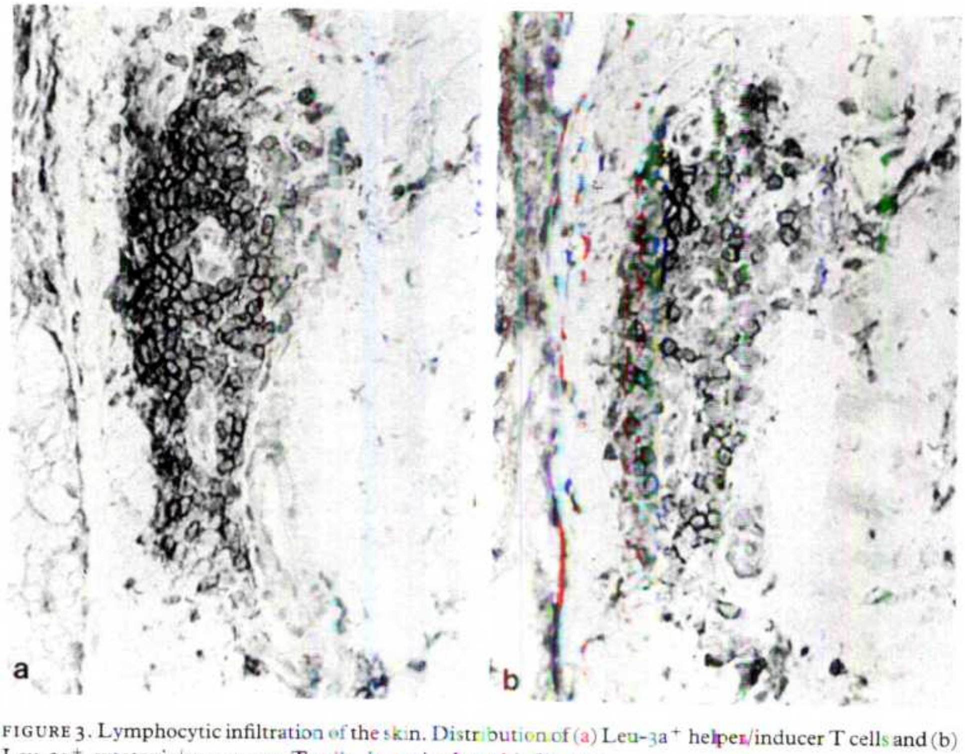

FIGURE 3. Lymphocytic infiltration of the skin. Distribution of (a) Leu-3a$^+$ helper/inducer T cells and (b) Leu-2a$^+$ cytotoxic/suppressor T cells, in peri-adnexal infiltrates.

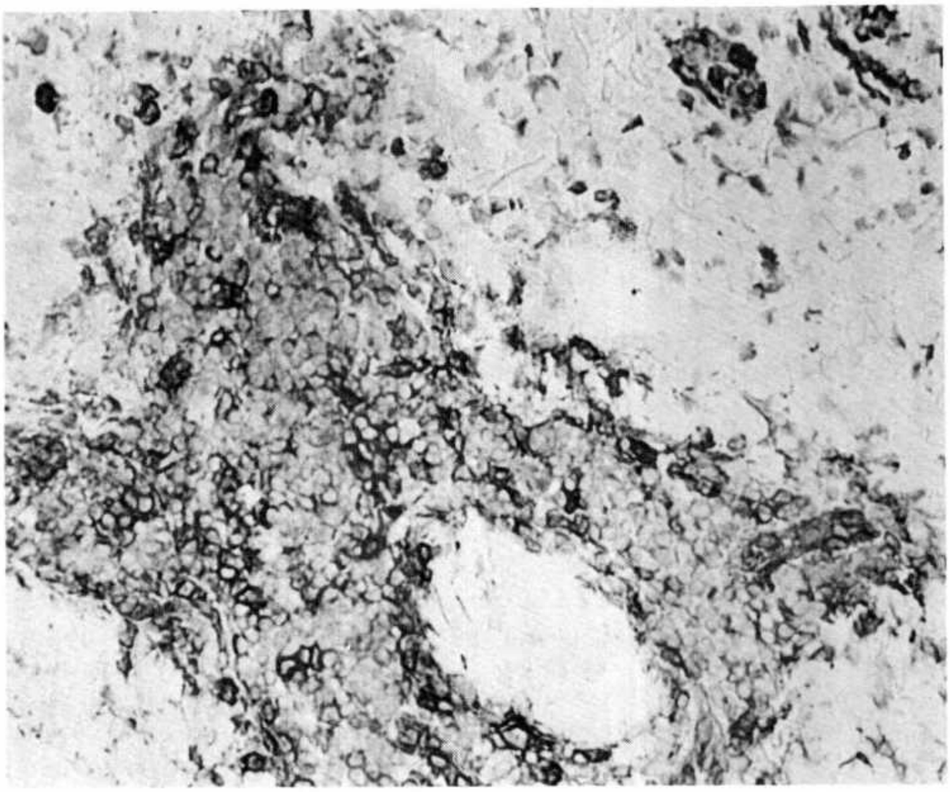

FIGURE 4. Lymphocytic infiltration of the skin. Staining with anti-HLA-DR antiserum, showing scattered HLA-DR⁺ macrophages. The large majority of the lymphoid cells appear negative.

TABLE 2. Results of staining with monoclonal antibodies in six patients with lymphocytic infiltration of the skin

Patient	Leu-1	Leu-2a	Leu-3a	TA-1	HLA-DR	OKT6
1	+ + + +	+ +	+ + +	+ + + +	+ +	–
2	+ + +	+	+ + +	+ + +	+ +	–
3	+ + + +	+	+ + + +	+ + + +	+ +	–
4	+ + + +	+ +	+ + +	nd	+ +	–
5	+ + +	+ +	+ + +	+ + +	+ +	–
6	+ + + +	+ +	+ + +	+ + + +	+ +	occ

Percentages semiquantitatively estimated from total number of infiltrating cells: occ = occasional cell; ± = 1–5%; + = 6–15%; + + = 16–30%; + + + = 31–60%; + + + + = more than 60%; nd = not done.

and/or related cells, were observed in the periadnexal, but not in the perivascular infiltrates. In the epidermis and adnexal epithelia normal numbers of HLA-DR⁺ and OKT6⁺ cells were found as compared with control sections from healthy donors.

The percentages of B cells as defined by a positive staining for IgM, or kappa or lambda light chains, were low (5% or less). In all patients cells reactive with OKM1 antiserum were only occasionally observed.

DISCUSSION

In the present study we have demonstrated that the perivascular infiltrates of lymphocytic infiltration of the skin (LIS) are predominantly composed of T lymphocytes. In addition strongly HLA-DR$^+$ macrophages were found scattered among the lymphoid cells. However, B lymphocytes as defined by a positive staining for surface immunoglobulins were few or completely absent. These findings are important since they provide criteria to differentiate LIS from lymphocytoma cutis. For many years the relationship between these conditions has been a matter of considerable debate. Whereas some investigators considered LIS to be a variant of lymphocytoma cutis (Postma & Sluiter, 1958), others felt that these conditions represent distinct entities, which can be distinguished clinically and histologically (Calnan, 1957; Wolf, 1957). However, in a recent report, in which the clinical and histological data of fifteen patients with LIS and thirteen patients with lymphocytoma cutis were reviewed (Lange Wantzin *et al.*, 1982), it was clearly demonstrated that clinical and histological criteria are insufficient to distinguish these conditions. Immunological (Braun-Falco & Burg, 1975) and ultrastructural (Schmoeckel *et al.*, 1977) studies have demonstrated a predominance of B lymphocytes in skin lesions of lymphocytoma cutis. Subsequently both conditions have been regarded as pseudo-B-cell lymphomas (Burg *et al.*, 1982). However, immunohistochemical studies supporting a B-cell origin of the lymphoid cells in LIS have not been reported. Two recent reports have shown that the large majority of the lymphoid cells in the perivascular infiltrates of LIS are T lymphocytes. In one of these studies (Konttinen *et al.*, 1981) the presence of an intracytoplasmic dot-like acid alpha-naphthyl acetate esterase activity was used as a T-cell marker. In the other study (David *et al.*, 1980) an anti-T-cell serum was prepared from horse anti-human lymphocyte serum by four successive absorptions with lymphocytes from patients with B-CLL. Immunofluorescence studies showed a predominance of T lymphocytes in the dermal infiltrates of eleven patients with LIS, but also in two patients with lymphocytoma cutis. However, detailed clinical and histological information on these patients was not given. The results of the present study clearly indicate that LIS represents a T-cell lymphoproliferative disease, which, unlike lymphocytoma cutis, cannot be regarded as a pseudo-B-cell lymphoma. It can also be concluded that immunohistochemical studies, which are often used to differentiate between benign and malignant lymphocytic infiltrates of the skin, are also necessary to achieve a correct classification of the different types of cutaneous lymphoid hyperplasia.

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Dermal elastosis in hairless mice after UV-B and UV-A applied simultaneously, separately or sequentially

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SUMMARY

The elastosis-inducing capacity of UV-A and UV-B was studied in the skin of hairless mice. After irradiation with UV-B and UV-A applied either simultaneously, separately or sequentially, the degree of elastosis in the dermis was microscopically evaluated. This semi-quantitative method showed no significant elastosis in the UV-A irradiated mice, and moderate elastosis in the UV-B irradiated mice. Heavy elastosis was observed when the mice were exposed sequentially to UV-B and a large dose of UV-A, but if a moderate dose of UV-A was given simultaneously with UV-B, the degree of elastosis was slightly reduced compared with the elastosis induced by UV-B alone.

Chronic sunlight exposure can induce degenerative changes in human skin (Lund & Sommerville, 1957) which produce visible wrinkles and laxity. Histopathologically, the most conspicuous feature is the basophilic degeneration or actinic elastosis in the connective tissue of the dermis. Changes histochemically identical to actinic elastosis have been produced in animal models by means of ultraviolet irradiation (Sams, Smith & Busk, 1964), but the action spectrum is unknown (Parrish *et al.*, 1978).

The aim of the present investigation was to study the elastosis-inducing capacity of UV-A (320–400 nm) and UV-B (280–320 nm) applied either separately, simultaneously or sequentially to different groups of lightly pigmented hairless mice. The sequential irradiation was designed to simulate the irradiation conditions of the human sunbed-user.

METHODS

Animals

Lightly pigmented, hairless, female mice of the Oslo/Bom inbred strain (275 hr/hr, from

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TABLE 1. Irradiation schedules

Group	Irradiation equipment	Filtration (2 mm glass)	Irradiation time: Daily (min)	Irradiation time: Weekly (days)	Irradiation time: Total (months)	Irradiance at skin level (mW/cm²): UV-B	Irradiance at skin level (mW/cm²): UV-A
1	0	0	0	0	0	0	0
2	1 sunlamp +						
	2 black lights	−	10	5	3	0·5	1·6
3	1 sunlamp	−	10	5	3	0·45	0·18
4	4 black lights	+	10	5	3	0	2·2
5	2 black lights	−	10	5	3	0·05*	1·4
	and subsequently						
	3 black lights	−	120	4	6	0·08*	2·2
6, 7, 8	1 sunlamp +						
	2 black lights	−	10	5	3	0·5	1·6
	and subsequently				2 (gr. 6)		
	3 black lights	−	120	4	4 (gr. 7)	0·08*	2·2
					6 (gr. 8)		
9, 10, 11	1 sunlamp +						
	2 black lights	−	10	5	3	0·5	1·6
	and subsequently				2 (gr. 9)		
	4 black lights	+	120	4	4 (gr. 10)	0	2·2
					6 (gr. 11)		

* The irradiance in the region 310–320 nm emitted by the unfiltered black light tubes.

Bomholtgaard, Denmark) were divided into eleven groups of twenty-five mice each (Table 1). Forty-five mice (Table 2) were excluded from the experiment, either because they died (seventeen mice) during the irradiation period or because they were sacrificed (twenty-eight mice) in order to follow the development of amyloidosis. The experimental irradiation started when the animals were 12 weeks old after 6 weeks of acclimatization in the laboratory.

Irradiation

Unfiltered UV-A irradiation was obtained from three black-light tubes (Philips TL 40 W/09), and filtered UV-A from four of these tubes. Filtration through specially selected window glass of 2 mm thickness eliminated the wavelengths below 320 nm (Staberg *et al.*, 1984). To keep the irradiance constant (2·2 mW/cm²) the distance from the tubes to the backs of the mice was 20 cm and 25 cm in the 'unfiltered' and 'filtered' groups, respectively. The UV-B irradiation was obtained from one Westinghouse sunlamp (40 W FS40) with an irradiance of 0·45 mW/cm² in the UV-B range and 0·18 mW/cm² in the UV-A range at skin level, 20 cm from the tube.

The combined UV-A and UV-B irradiation ('artificial UV-sunrays') was designed to simulate the ultraviolet portion of natural sunlight (Wulf *et al.*, 1982). The light source was a bank of one Westinghouse sunlamp and two black light tubes. The irradiances at skin level, 20 cm from the tubes, were 0·5 mW/cm² in the UV-B range and 1·6 mW/cm² in the UV-A range.

The spectrum was measured by an IL 700 research radiometer with detector PT 171C/297 (International Light Inc.) and a Jobin Yvon monochromator in front of the detector. By means of the monochromator, 2-nm sections were scanned to compose the spectrum of the irradiation. The irradiances in the UV-A and UV-B ranges were calculated from the total emitted irradiance (W/cm^2) and the irradiances measured with a WG 320 (1·5 mm thick) and a Wratten 2B (Kodak) filter in front of the detector. The measurements were made in the cages under the same conditions as during irradiation of the mice (i.e. no shielding as food and water equipments were removed during irradiation).

Experimental procedure

Five mice were kept in each cage with free access to standard laboratory food and water. They were irradiated in their cages without any form of shielding, according to the irradiation schedules shown in Table 1. The total energy received in each group is shown in Table 2. The irradiation was started simultaneously in all groups, and the experiment went on for 58 weeks including the post-irradiation observation time. The mice were then sacrificed for histopathological and histochemical studies.

One strip of dorsal skin, 4 cm long, at right angles to the long axis outside tumour-bearing areas, was removed from each mouse, fixed in 4% buffered formaldehyde, embedded in paraffin and sectioned at 7 μm. The stains were: haematoxylin and eosin (H & E) Luna's aldehyde fuchsin for elastic fibres (Kligman, 1981), and Congo Red for amyloid deposits. The elastosis was estimated microscopically in two sections of each skin sample. First, all sections were reviewed to define the relative amount of elastic tissue in normal non-irradiated mouse skin as well as in irradiated mouse skin. The degree of elastosis was estimated on an arbitrary scale from 0 to 3, where degree 0 represented no elastosis (Fig. 1) and degree 3 the heaviest form of actinic elastosis (Fig. 4). Degrees 1 and 2 represented the intermediate steps, with slight and moderate

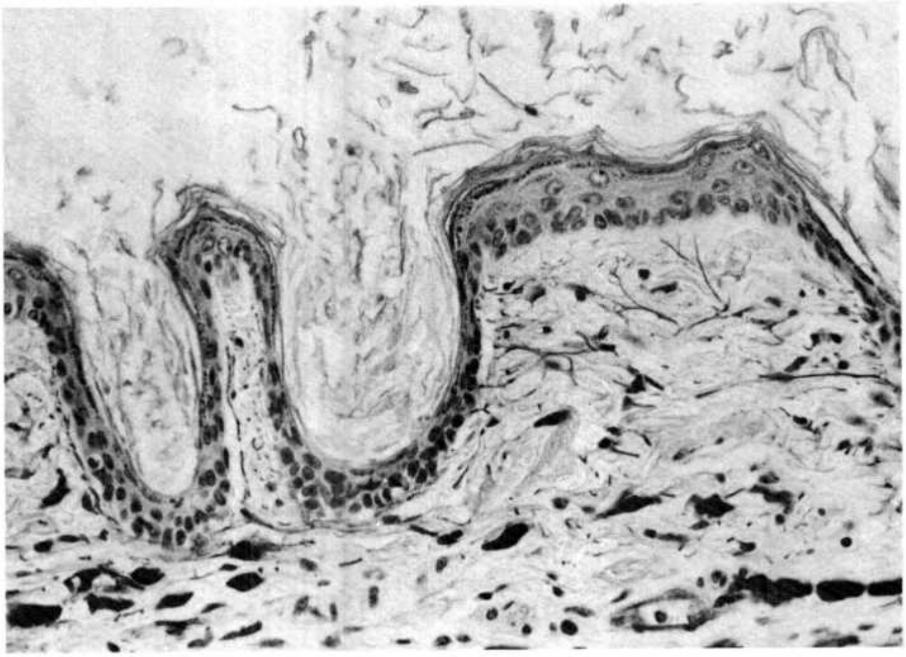

FIGURE 1. Section of non-irradiated mouse skin with fine elastin strands in the dermis and normal epidermal thickness (Luna's stain, × 400).

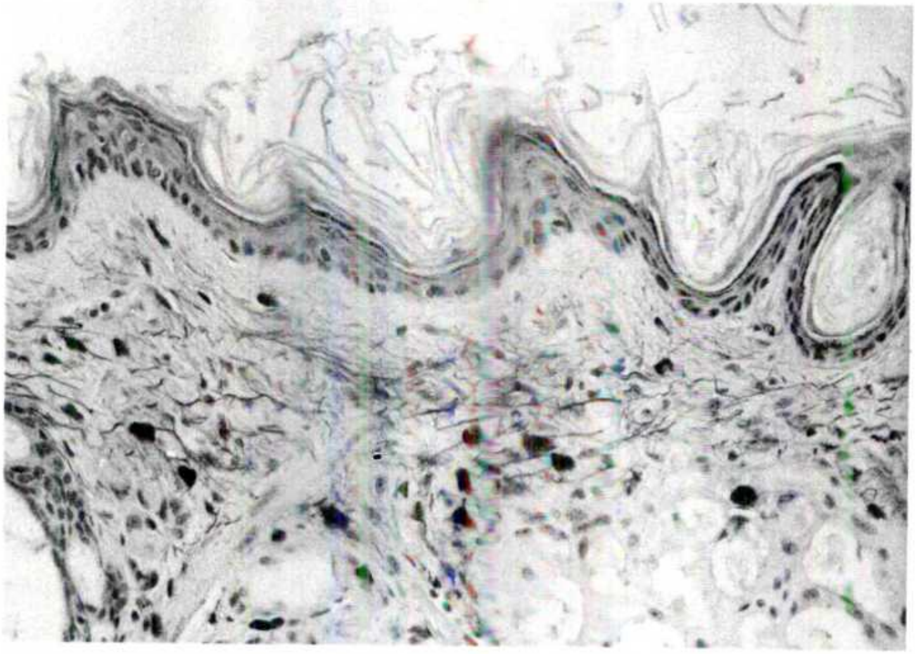

FIGURE 2. Section of irradiated mouse skin with slight elastin hyperplasia (Luna's stain, × 400).

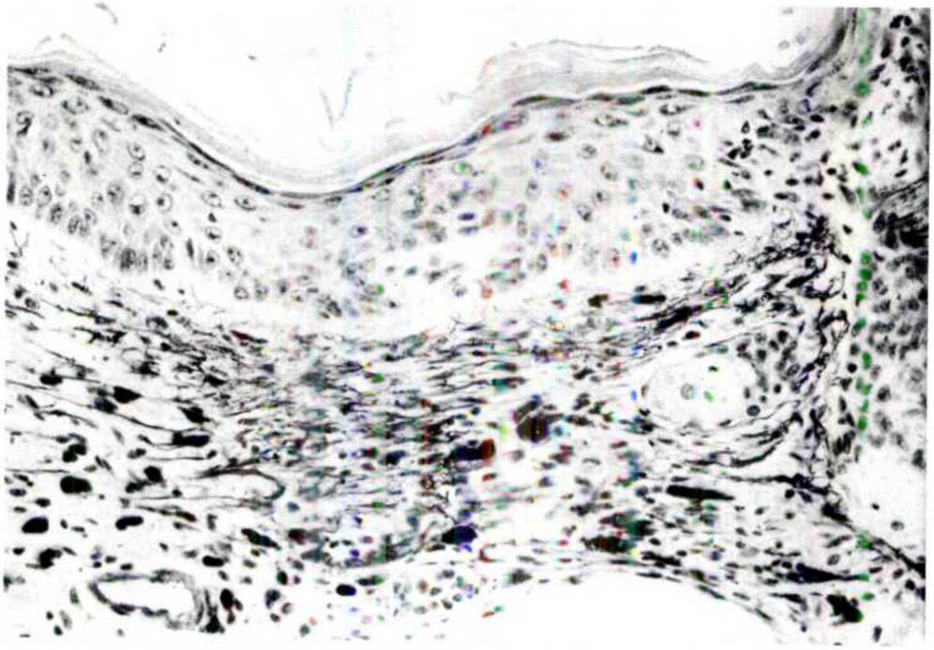

FIGURE 3. Irradiated mouse skin with moderate elastin hyperplasia and moderate epidermal hyperplasia (Luna's stain, × 400).

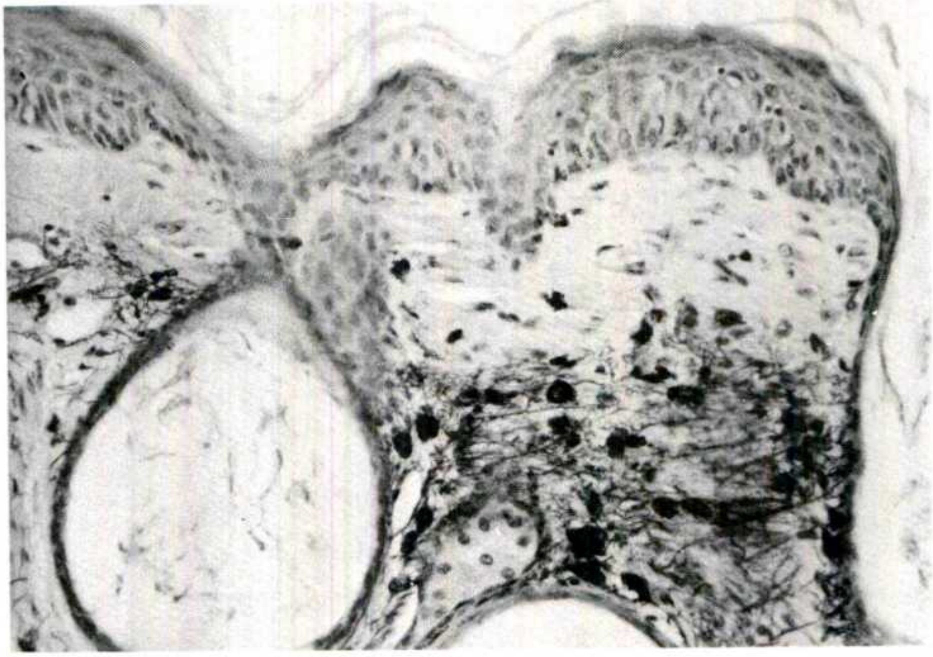

FIGURE 4. Irradiated mouse skin with heavy elastin hyperplasia and severe epidermal hyperplasia (Luna's stain, × 400).

elastosis (Figs 2 and 3). The estimation was done blind. As our semi-quantitative method was rather insensitive to slight changes, and as there was no statistical difference between the degrees 0 and 1 and the degrees 2 and 3, these were pooled, respectively (Table 2).

The results were analysed by the chi-square test with Yates' correction.

RESULTS

The mice in the groups exposed to 'artificial UV-sunrays' (groups 2 and 6–11) or only to UV-B (group 3) experienced a slight initial erythema followed by transient pigmentation, but were never burnt. UV-A alone induced neither erythema nor pigmentation.

The actinic elastosis appeared in the upper dermis as elastin hyperplasia, seen as overgrowth of tangled and thickened fibres with elastic tissue staining properties, but never reaching the stage of amorphous degeneration seen in badly damaged human skin. The incidences of elastosis (number of mice with degree 2–3 elastosis/total number of mice) in each group are given in Table 2.

There was no elastosis (degree 2 or 3) in the controls. Group 2 ('artificial UV-sunrays'), group 4 (filtered UV-A), and group 5 (unfiltered UV-A) did not show significant elastosis compared with the controls, but in group 3 (UV-B) the incidence of mice with elastosis was significantly higher than that observed in the controls ($P < 0{\cdot}05$). In groups 6–8 ('artificial sunrays' followed by UV-A) and groups 9–11 ('artificial sunrays' followed by filtered UV-A) the incidences of elastosis were significantly higher than those observed in the controls ($P < 0{\cdot}001$). When compared with the mice exposed solely to 'artificial sunrays' (group 2) we found a significantly

TABLE 2. Groups of mice, total energy applied in the UV-B and UV-A range, and degrees of elastosis with significance specifications

	Cumulative energy (J/cm²)		Elastosis (degree 2 + 3), total					
Group	UV-B 280–320 nm	UV-A 320–400 nm	No. of mice	%		*P* values when compared with controls	*P* values when compared with group 2	
1	0	0	0/21	0			n.s.	
2	20	63	1/23	4		n.s.		
3	19	7	5/21	24		0·05	n.s.	
4	0	70	1/24	4		n.s.	—	
5	43 (43)*	1765	1/21	5		n.s.	—	
6	34 (14)*	633	3/18	17	41	0·001	n.s	0·005
7	48 (28)*	1203	11/20	55			0·001	
8	62 (42)*	1773	11/23	48			0·005	
9	20	633	13/19	68	56	0·001	0·0005	0·0005
10	20	1203	8/19	42			0·01	
11	20	1773	12/21	57			0·0005	

* The amount of energy in the region 310–320 nm emitted from the unfiltered UV-A tubes is given in parentheses.

TABLE 3. The relationship between the degrees of elastosis and skin cancer induced by UV-irradiation

Degree of elastosis	Mice with tumours No.	Mice with tumours (%)	*P* values when compared with no elastosis	*P* values
0	7	(8)		
1	26	(26)	0·005	0·0001
2	43	(80)	0·0001	n.s.
3	11	(85)	0·0001	

higher incidence of elastosis in groups 6–8 ($P < 0{\cdot}005$) and in groups 9–11 ($P < 0{\cdot}0005$). The incidence of elastosis in groups 6–8 (41%) was slightly lower than that observed in groups 9–11 (56%), but the difference was not significant.

About 40% of all the animals developed skin cancers. The carcinogenic effect of the irradiation is described elsewhere (Staberg *et al.*, 1983, 1984). The tumours and the actinic elastosis seemed to develop in the same animals with a very high degree of correlation (Table 3).

General screening for amyloidosis revealed amyloid deposits in the skin of 61% of all mice. This showed no correlation with the applied irradiation, but developed with increasing age of the animals.

DISCUSSION

In 1957 Lund & Sommerville performed a semi-quantitative evaluation of elastosis (basophilic degeneration) in exposed human skin. To our knowledge, however, quantification has not yet been used in evaluating actinically induced elastosis in animals. Previous investigations have focussed on the quality rather than the quantity of the degenerative processes (Berger, Tsambaos & Mahrle, 1980; Kligman, Akin & Kligman, 1982). When studying the different degenerative alterations in the irradiated mouse skin, we found that the elastosis was the most conspicuous feature (in Luna's stained sections), and the only variable suitable for semi-quantitative evaluation.

Berger *et al.* (1980) found elastosis in the skin of albino hairless mice after irradiation with high doses of filtered (5 mm glass) UV-A from black light tubes (65 J/cm UV-A + 0·02 J/cm^2 UV-B daily for 400 days). We could not demonstrate any significant elastosis after smaller doses of UV-A (group 4—filtered UV-A for 3 months; group 5—unfiltered UV-A for 9 months) (Tables 1 and 2). This discrepancy might be due to differences in the experimental approach, especially in the irradiation doses and the different pigmentation of the experimental animals. Group 5 did not show significant elastosis, although this group received a rather high total energy both in the UV-A and the UV-B range of the spectrum. The UV-B irradiation was, however, delivered in wavelengths between 310 and 320 nm, which obviously are ineffective in producing elastosis in hairless mice in the applied dose (Table 2).

In group 2, which was irradiated with a combination of UV-A and UV-B simultaneously ('artificial UV-sunrays' for 3 months), we found no significant elastosis. However, irradiation with 'artificial UV-sunrays' for 3 months followed by UV-A (either unfiltered (groups 6–8) or filtered (groups 9–11) for 2–6 months induced highly significant elastosis as compared with group 2 or the controls. This indicates that although UV-A in itself is a poor producer of elastosis, it seems to augment significantly the effect of combined UV-B and UV-A in exposed skin. The spectrum and daily energy output of our UV-A source (16 J/cm^2) corresponded to the energy received during a usual human UV-A sunbed exposure for cosmetic reasons (15–20 J/cm^2) (Greaves & Vella Briffa, 1981). As the 'artificial UV-sunray' irradiation and subsequent UV-A irradiation simulates the conditions of the human sunbed-user, it may be anticipated that natural sun exposure followed by exposure in a UV-A solarium might not be innocuous to the human skin.

It is surprising that group 3 (UV-B), unlike group 2 (UV-A + UV-B), showed significant elastosis compared with the controls (Table 2), but Forbes, Davies & Urbach (1978) have observed that small doses of UV-A have a similar effect in decreasing the carcinogenic effect of UV-B. Our results might be due to a screening effect of epidermal hyperplasia induced by UV-A (Willis, Menter & Whyte, 1981; van der Leun & van Weelden, 1983; Staberg *et al.*, 1983b).

Both epidermal carcinogenesis and dermal actinic elastosis are induced by UV-irradiation, and we found a high positive correlation between these changes (Table 2). Whether there is any causal connection is uncertain.

In a previous study, Wulf *et al.* (1982) observed amyloidosis in the skin of hairless mice irradiated with UV-B + UV-A. A general screening for amyloid deposits in all the animals in the present study revealed that 61% of the mice had developed general amyloidosis with deposits in the dermis and in the internal organs. The amyloidosis showed no correlation with the irradiation, but a high positive correlation with the age of the animals.

We conclude that irradiation with UV-A alone in the doses we used does not induce significant elastosis in the skin of hairless mice, that UV-B does induce elastosis, and that UV-A applied after a period of pre-irradiation with combined UV-B and UV-A does enhance the elastosis-inducing effect of UV-B. However, it seems that when UV-A in small doses is given simultaneously with UV-B, the degree of elastosis observed with UV-B alone is reduced. Whether the results can be extrapolated to human skin is debatable, but they support the suspicion that UV-A irradiation in large doses is not as innocuous as is often claimed.

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Elastic fibres: histological correlation with orcein and a new monoclonal antibody, HB8

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SUMMARY

HB8 is a new monoclonal antibody, prepared against human lymphocytes, which has been found to react with elastic fibres, possibly the microfibrillar component.

Samples of normal human and animal skin and human skin from various pathological conditions were subjected to immunolabelling with HB8, and alternate sections from each sample were stained by a standard orcein procedure. The individual fibres were clearly marked by the HB8 and this was confirmed by comparison with the standard elastic tissue stain. HB8 provides a new direct, practical and accurate method for the study of elastic tissue.

Elastin is an insoluble protein complex present in most connective tissues. It constitutes approximately 4% of the dry weight of skin, where it forms a complex and extensive network in the dermis. It is formed mainly by smooth muscle cells but may also be synthesized by endothelial cells, ligamentum nuchae fibroblasts and fibrocartilaginous chondroblasts. There are two major components of elastic fibres (Ross, Fialkow & Altman, 1977; Varadi, 1976). A central amorphous material is surrounded by a microfibrillar scaffolding or envelope. The amorphous material constitutes 90% of the mature elastic fibre (Ross *et al.*, 1977), becoming more prominent with advancing age. The microfibrils are first noticed during early fetal development. They have a diameter of 10–12 nm and appear to be devoid of any mechanical function in their isolated state.

The most immature forms of elastic fibre are found in the papillary dermis; these consist of little more than bundles of microfibrils which extend from the basal lamina to the papillary dermis, often at right angles to the dermis. These fibres have been called oxytalan fibres (Gawlik, 1965); they help to anchor the basal lamina and form a template upon which elastin is secreted at deeper levels in the dermis. The oxytalan fibres branch to form a horizontal plexus in the upper reticular dermis. Small amounts of stainable elastic matrix are secreted into the

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microfibrillar protein in this region, which are then known as elaunin (Gawlik, 1965). Large mature bands or sheets of elastic fibres are restricted to the reticular dermis (Uitto, 1979; Uitto *et al.*, 1982).

We have recently produced a new monoclonal antibody (MCA), called HB8, by immunizing Balb c mice with human lymphocytes. It was also observed to react with elastic fibres, possibly the microfibrillar component. Serial samples of normal human and animal skin and skin from various pathological conditions were therefore subjected to immunolabelling with HB8, alternate sections being stained by a standard elastic tissue stain.

METHODS

A new MCA, HB8, was prepared according to the technique of cellular hybridization (Koehler & Milstein, 1975) by immunizing Balb c mice with a suspension of lymphoid cells obtained from the human thoracic duct (Brochier *et al.*, 1983). A variety of MCAs was obtained and these were subjected to a systematic study to determine the cross-reactivity between the anti-lymphocyte MCAs and human and animal tissue (Schmitt *et al.*, 1982; Yonish-Rouach *et al.*, 1982). A cell line secreting an IgG called HB8 was discovered to label elastic tissue (Brochier *et al.*, 1983). This was selectively cloned and studied.

Tissue specimens

These included normal adult human abdominal, axillary, breast and preputial skin, skin and lung sections from a 19-week human embryo and samples of rabbit and mouse lip.

Immunohistochemical procedure

Tissue specimens were snap-frozen in liquid nitrogen and 4-μm thick frozen sections were cut, air-dried and fixed in cold acetone (10 min).

Indirect immunofluorescence was performed as follows: sections were incubated with HB8 (dilution 1:50, 45 min, 37°C), and washed in phosphate-buffered saline (PBS; pH 7·2, 30 min). They were then incubated with fluorochrome-conjugated second layer antiserum (Meloy goat anti-mouse IgG, labelled with fluorescein isothiocyanate, dilution 1:20, 30 min, 37°C), washed as before, and then mounted in modified polyvinyl alcohol medium and examined under a Zeiss fluorescent microscope with epi-illumination.

Alternate sections from each biopsy were stained as follows: they were immersed in a bath of alcohol (10 min), then in orcein (12 hours at room temperature), then in 95° alcohol (10 min). The sections were washed in running water (5 min), stained with haemalum (10 min), washed again in running water (5 min), dehydrated by dipping in three baths of absolute alcohol for 1 min, passed through three baths of toluene, and mounted in Canada Balsam (Pro-texx).

To ensure the specificity of the reaction between HB8 and elastic fibres, immunofluorescent labelling using other MCA (OKT6, BL6, BL7 and D47) was performed in the same conditions as for HB8.

RESULTS

Bright green immunofluorescent staining was visible in the specimens under study. This followed the characteristic distributions of elastic tissue as confirmed by comparison with the orcein slides. The specimen did not react with OKT6, BL6, BL7 and D47.

The general topography of elastic tissue is shown in Fig. 1. Here the fine oxytalan fibres can be seen to be mounting towards the basal layer to form a complex interlacing network. Examination

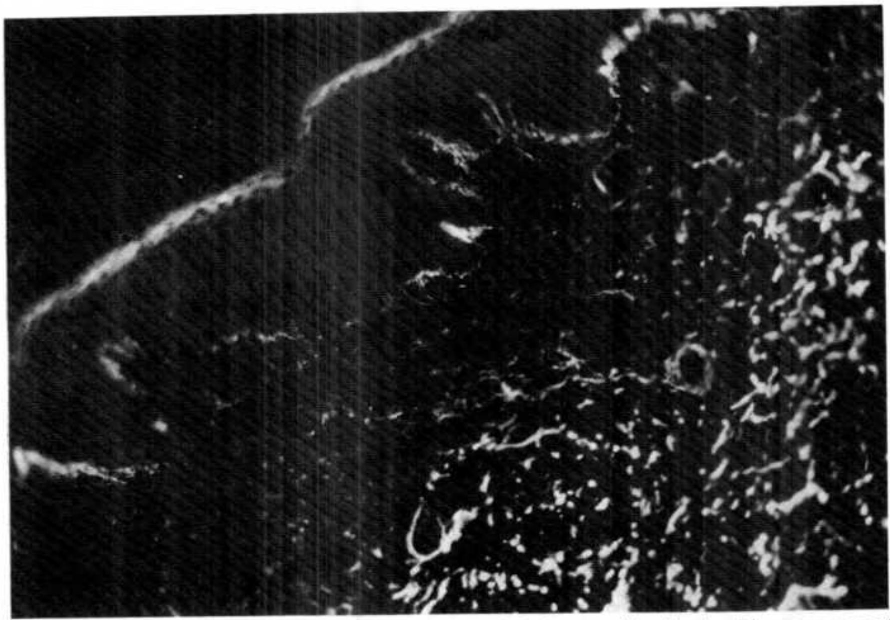

FIGURE 1. Comparative histological aspects of staining of the human skin elastic with a new monoclonal antibody (HB8) and with orcein. General topography of elastic tissue stained with HB8 (× 250).

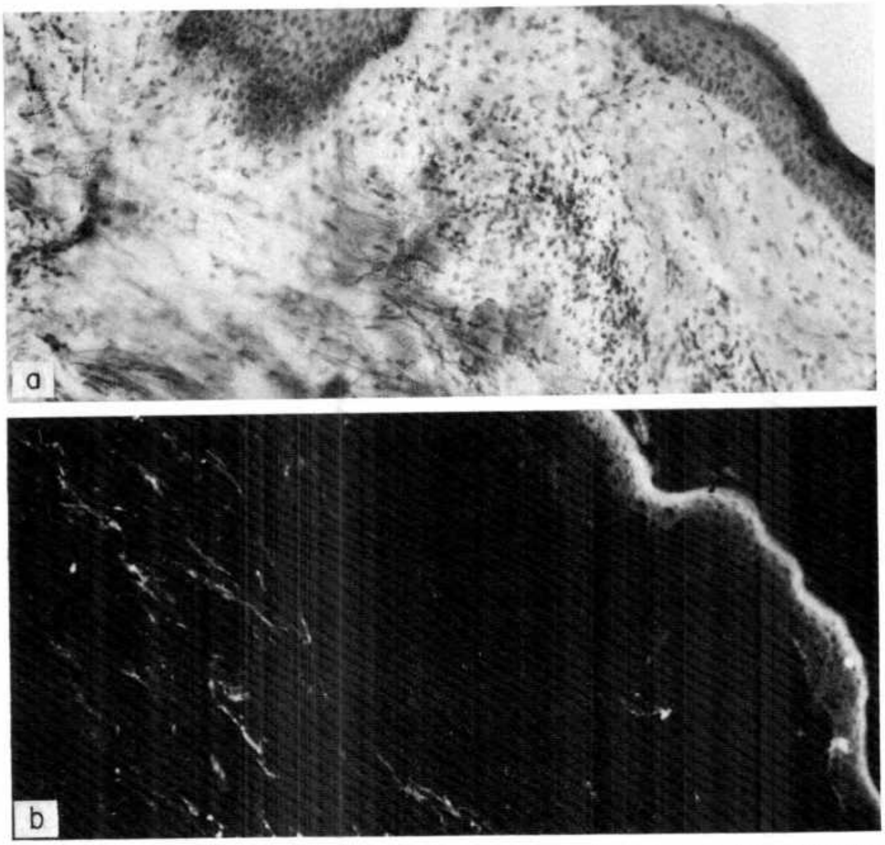

FIGURE 2. Comparative staining of elastic tissue in a case of giant haemangioma. (a) Orcein. (b) HB8. (× 100.)

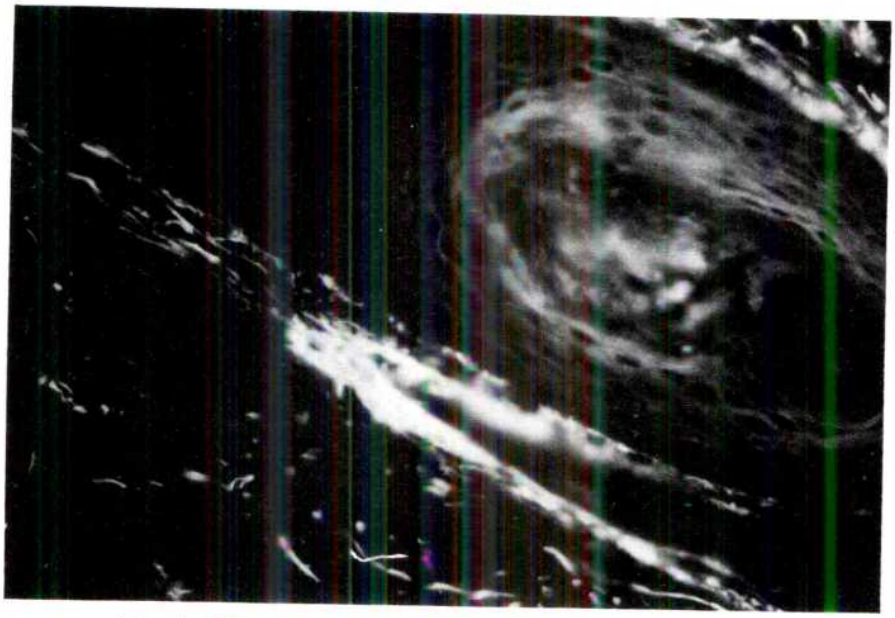

FIGURE 3. A longitudinal section of a hair follicle showing the elastic tissue sheath (× 400).

of the dermis reveals sheets of elastic tissue travelling in different planes. The fibres cut in cross-section produce the typical appearance of pseudofragmentation. At a deeper level in the dermis, the fibres become thicker (Fig. 1). The internal elastic laminae of two large blood vessels are also clearly labelled.

Large sheets of elastic tissue running at right angles to each other can be seen (Figs 1 and 2). This can be compared with the orcein staining.

Figure 1 demonstrates transverse sheets of elastic tissue in the dermis and the fine fibres communicating with the basal layer.

The elastic tissue in the blood vessels (from normal human prepuce) was also labelled.

A longitudinal section of a hair (Fig. 3) shows the elastic tissue sheath of the hair follicle. A condensation of elastic tissue is present (? Arao–Perkins body).

The branching characteristic of elastic fibres is visible in Fig. 4b (deep dermis of solar elastosis). Figure 4a shows the same features in orcein staining. A view of the deep dermis from a phototest of normal human skin demonstrates that the elastic fibres appear to be stained only on the periphery (Fig. 5). Some fibres are striated and others, cut in cross-section, are annular.

These features suggest that it is the microfibrils which have been labelled and not the central amorphous material.

A group of blood vessels from an angioma (giant haemangioma, Fig. 6) demonstrate labelling of the internal elastic lamina (IEL). There appears to be reduplication of the IEL in some vessels.

Samples of rabbit and mouse lip were also studied. The rabbit lip had little elastic tissue, as shown in both HB8 and orcein preparations, but the mouse lip showed a dense concentration of elastic tissue with both techniques.

Tissue from a 19-week-old fetus was found to be negative with standard orcein stain but with HB8 fine microfibrillar staining was noted in the connective tissue surrounding the hair follicles

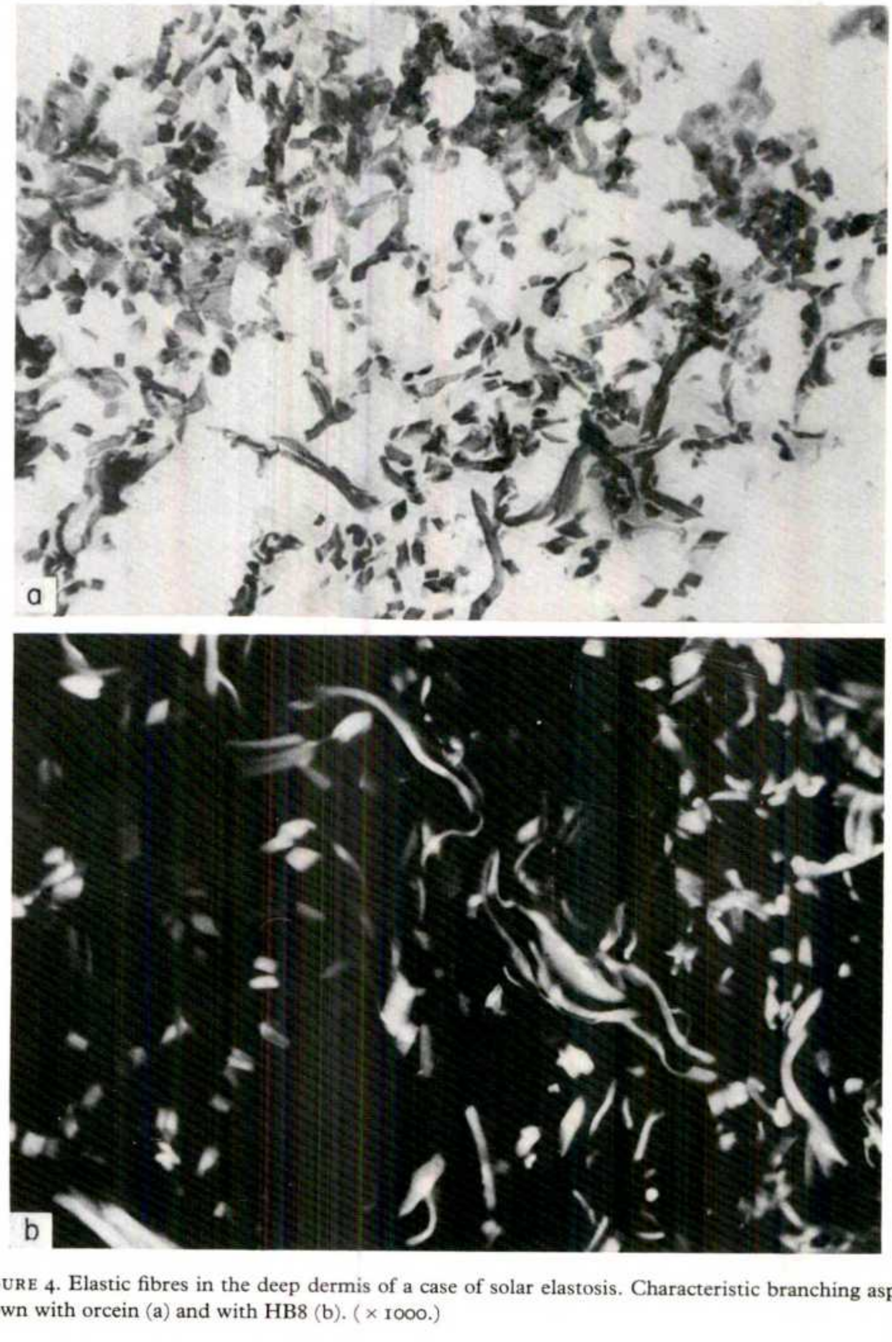

FIGURE 4. Elastic fibres in the deep dermis of a case of solar elastosis. Characteristic branching aspect shown with orcein (a) and with HB8 (b). (× 1000.)

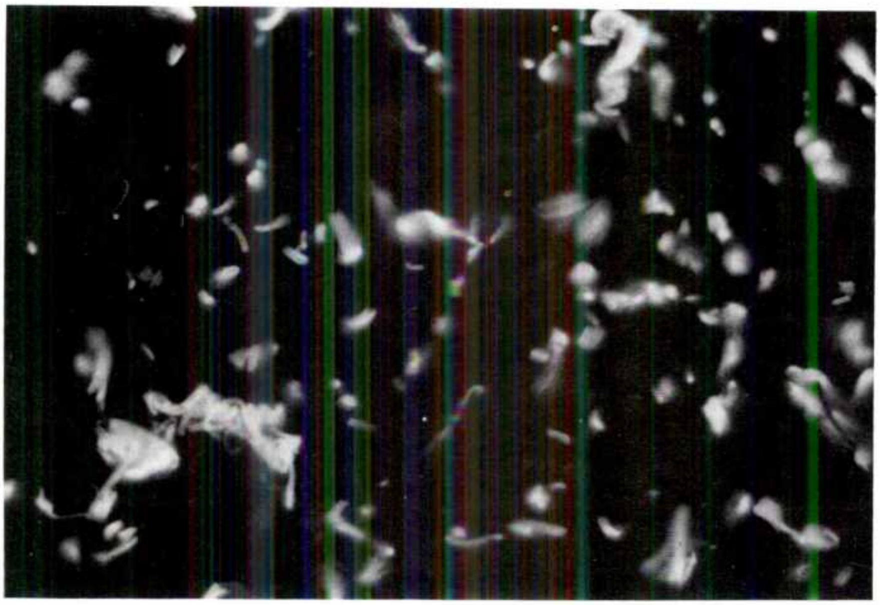

FIGURE 5. The deep dermis in a phototest of normal skin, showing the microfibrillar pattern of the staining of the elastic fibres with HB8 (× 1000).

and at some points in the dermis. Fine microfibrillar-type labelling was also clearly observed beside developing bone in a section of finger.

Many samples of solar elastosis were studied (confirmed by the orcein stain). The upper oxytalan fibres were still in evidence beneath the basal layer, and the clumps of elastic tissue in the upper and middle dermis were markedly stained with HB8.

A skin biopsy from a case of pseudoxanthoma elasticum showed the characteristic tangled-wool appearance with both orcein and HB8 staining.

DISCUSSION

HB8 is a new MCA prepared against human lymphocytes. It appears to react with the microfibrillar scaffolding of the elastic fibre and is the first MCA to react with elastic tissue. This highlights the intriguing phenomenon of cross-reactivity between human lymphocytes and cutaneous tissue (Kanitakis *et al.*, 1983). We believe it labels an antigen common to both lymphocytes and the microfibrillar component of elastic tissue, and further study is being undertaken at both electron microscopic and histochemical levels to ascertain this (Thivolet *et al.*, 1983).

It provides a unique and practical tool having obvious daily application for the anatomist, histopathologist and comparative biologist. It will be of particular interest to cardio-respiratory researchers studying emphysema and atherosclerosis and to workers studying the ageing process (Ryhanen & Uitto, 1982; Sandberg *et al.*, 1982).

It is easier to use than orcein, takes less time and reacts directly with the elastic fibre, whereas fixation and staining with orcein may produce artefactual changes in the elastic tissue under study (Jarrett, 1979a,b). It can also be used on frozen sections and studied under the light microscope using the immunoperoxidase technique with counter-staining with haematoxylin

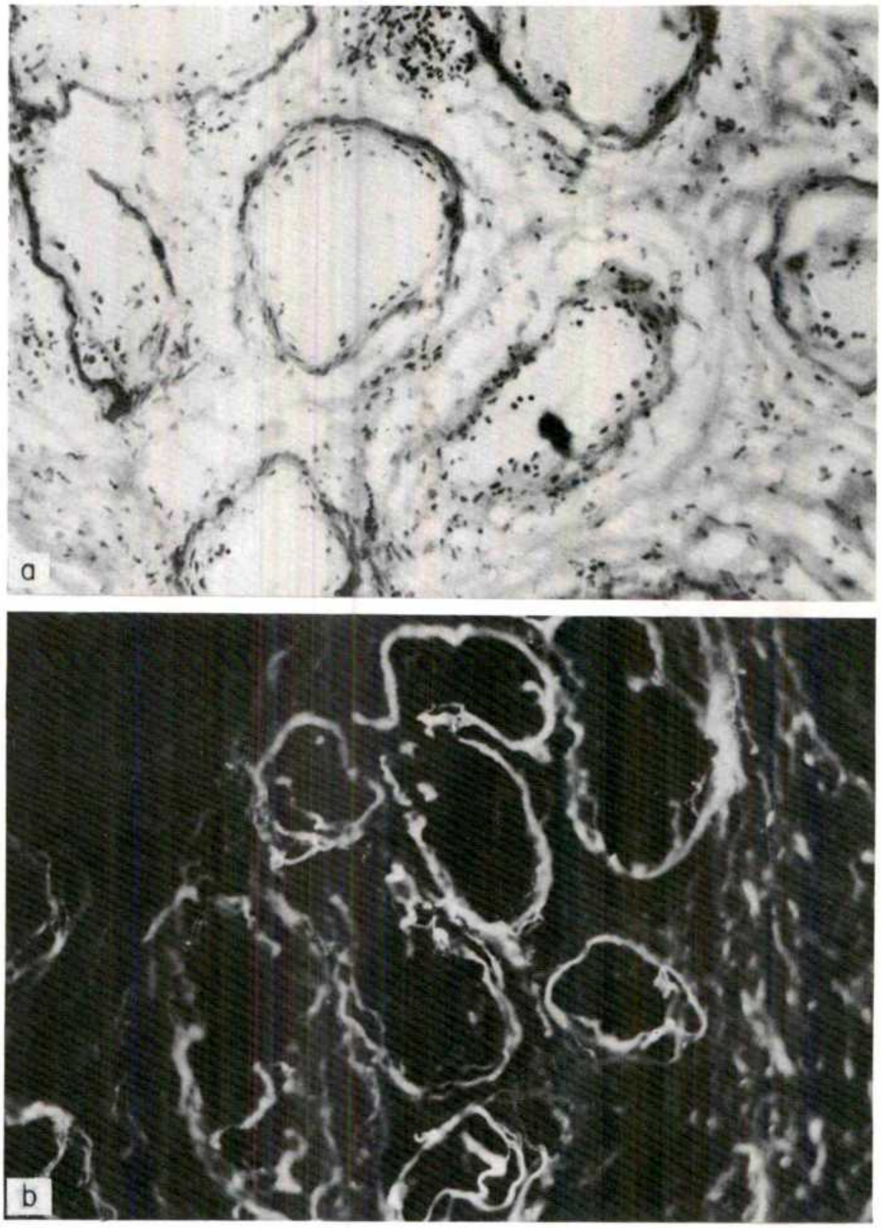

FIGURE 6. Staining of the elastic fibres of blood vessels in a case of giant haemangioma (b) and with orcein (a). (× 400.)

and eosin for direct *in situ* comparison between standard histopathology and indirect immunofluorescence.

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Transepithelial elimination of elastic fibres in the regenerated human epidermis

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SUMMARY

The elimination of elastic fibres through the regenerated epidermis of human skin was studied using histochemical, immunofluorescent and electron microscopic techniques. No abnormality was detected in the elastic fibres which were being eliminated, but they were in direct contact with the cytomembranes of some lymphocytes and were captured by histiocytes. It seemed that these elastic fibres were recognized as a foreign substance and provoked inflammation. Lymphocytes and histiocytes may play an important role in the transepithelial elimination of elastic fibres.

Elastic fibres are among the largest elements in the dermis to be eliminated transepithelially. Transepithelial elimination of elastic fibres occurs in pathological states such as elastosis perforans serpiginosa, perforating folliculitis, perforating pseudoxanthoma elasticum, keratoacanthoma, healing wounds, and discoid lupus erythematosus (Mehregan, 1970; Lund & Gilbert, 1976). In these cases, the eliminated elastic fibres may be either degenerate (Mehregan, 1970) or intact (Pinkus, 1965).

In the present study the elastic fibres in healing wounds were examined by various methods.

METHODS

Ten adult patients, who had sustained a third degree burn of the extremities approximately 1 month previously, were studied. The wound healing was incomplete when the specimen was taken. The specimens were taken from the regenerated skin at a distance of approximately 1 cm from the margin of the ulcer during surgical debridement. Each specimen was divided into three pieces.

1. Histochemistry. The first piece was fixed with 10% buffered neutral formalin solution for histochemical study. Sections were stained with haematoxylin and eosin, Pinkus' acid orcein-Giemsa, Unna's orcein, Verhoeff's iodine–iron haematoxylin, Weigert's resorcin-fuch-

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sin, Gomori's aldehyde–fuchsin, periodic acid–Schiff, Masson's trichrome, Congo Red and Victoria Blue stains.

2. Direct immunofluorescence (DIF). The second piece was snap-frozen in dry ice and acetone, and stored at −70°C until use. Four-micron sections were cut in a cryostat (Bright 5030 microtome) for DIF study. DIF staining was performed using commercial FITC-labelled goat anti-human IgG, IgM, IgA, C1q, C3 and C4 conjugates (Medical Biological Laboratory, Nagoya, Japan; F/P ratio 1·2 to 1·8; working dilution 1 : 15).

3. Electron microscopy. The third piece was cut to a size of approximately 1 × 1 × 2 mm and fixed for 3 hours in a 5% glutaraldehyde solution buffered to pH 7·4 with sodium cacodylate at 4°C. After an overnight rinse in the same buffer, they were postfixed for 1 hour with 1% osmic acid solution in the same buffer. Following staining with 1% uranyl acetate in 50% methanol, they were dehydrated through an ethanol–propylene oxide series using the routine technique for transmission electron microscopy and then embedded in Luveak 812. Thin sections were stained with uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). They were examined in Hitachi HU 12A and H-800 electron microscopes at 75 or 100 kV.

Control studies. Portions of normal skin to be used in skin grafting were obtained from the extremities of three adult patients and used as control specimens.

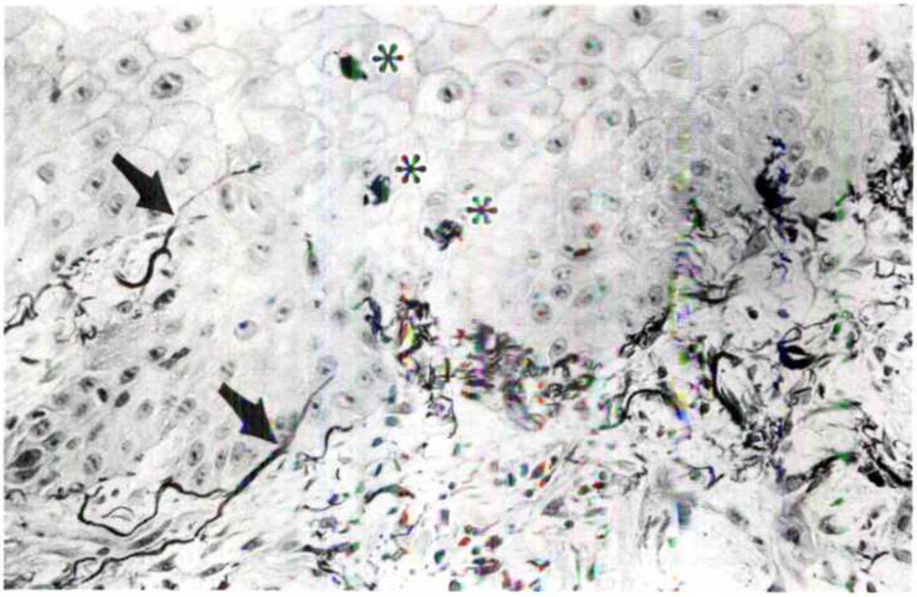

FIGURE 1. Weigert's resorcin–fuchsin stain. Aggregated as well as solitary elastic fibres are seen at the dermo-epidermal junction and the epidermis. On the left, two solitary elastic fibres (arrows) penetrate the dermo-epidermal junction from dermis into epidermis, and in the centre, three masses of aggregated elastic fibres (asterisks) are present in the epidermis. (× 340.)

RESULTS

Histochemistry (Fig. 1). The skin was composed of the regenerated epidermis, dermis and the newly formed granulation tissue. In the upper dermis elastic fibres were scattered loosely or accumulated massively in small circumscribed areas. Sometimes they were intermingled with inflammatory cells. When elastic fibres penetrated the dermo-epidermal junction from dermis into epidermis, they were present as a small aggregated mass or a solitary fibre. In the epidermis, they were still aggregated or solitary. Elastic fibres showed normal histochemical staining properties.

DIF. No elastic fibre had specific immunofluorescence, but they did show the usual autofluorescence.

Electron microscopy. There were elastic fibres in the upper dermis, where the newly formed collagen fibres were loosely arranged. The elastic fibres were approximately 0·8 to 3 μm thick and they were fusiform in cross section. Skeleton fibrils and dense granular elements of elastic fibres (Hashimoto & DiBella, 1967) appeared normal. Elastic fibres were seen to be scattered diffusely, aggregated massively or enveloped with the cytomembrane of histiocytes. Aggregated elastic fibres were intermingled randomly with inflammatory cells, especially lymphocytes and histiocytes. Occasionally they were in direct contact with the cytomembranes of a number of

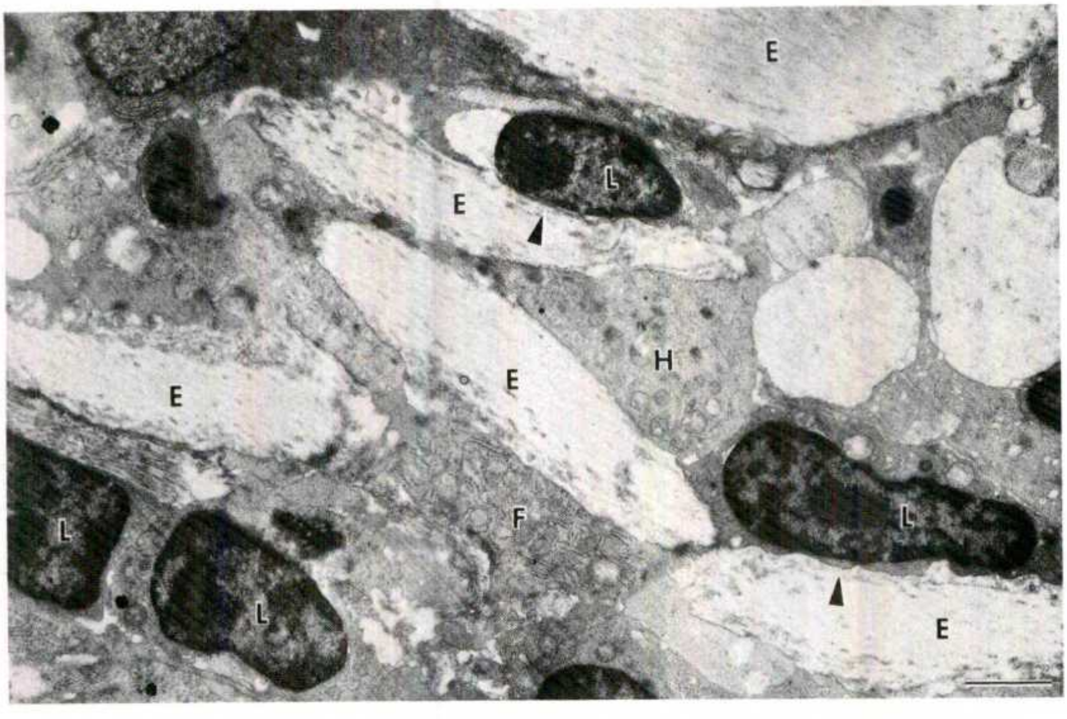

FIGURE 2. Elastic fibres (E) intermingled with inflammatory cells, especially lymphocytes (L). Elastic fibres are in contact directly with the cytomembranes of lympocytes (arrowheads). F = fibroblast, H = histiocyte. Bar = 2 μm. (× 5700.)

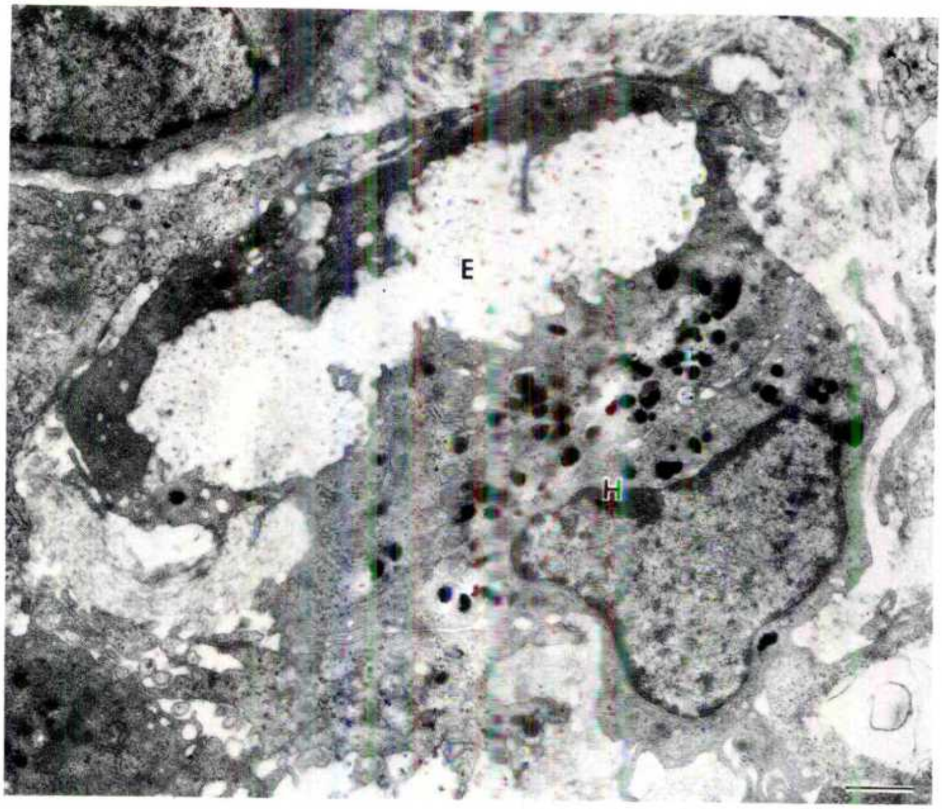

FIGURE 3. An elastic fibre (E) is enveloped by the cytomembrane of a histiocyte (H), which has numerous dense bodies. Bar = 1 μm. (× 8900.)

lymphocytes (Fig. 2). When elastic fibres were enveloped by the cytomembrane of the histiocytes, there was no space between the surface of the fibres and the cytomembrane of the histiocytes (Fig. 3). However since serial sections were not examined, we could not confirm whether or not an entire elastic fibre was phagocytosed by the histiocyte.

At the dermo-epidermal junction, elastic fibres were still accompanied by lymphocytes and histiocytes (Fig. 4A). The surface of the elastic fibres was entirely or partially enveloped by the cytomembrane of the histiocytes (Fig. 4B). Basal keratinocytes as well as lower spinous cells were compressed where elastic fibres penetrated the basal lamina of epidermis (Fig. 4A), and the organelles of those keratinocytes were indistinct.

Elastic fibres were seen throughout the thickness of the epidermis. They were located in the intercellular spaces between keratinocytes and their ultrastructural appearance was normal. They were sometimes accompanied by lymphocytes and histiocytes (Fig. 5A). The keratinocytes were compressed by elastic fibres and often formed hemidesmosomes with a sub-basal dense layer along the surface of the elastic fibres (Fig. 5B).

In the horny layer, the elastic fibres were randomly oriented. Their thickness was almost the same as that in the dermis. However, sometimes they were cleaved transversely, and the amount of dense granular substance of elastic fibres (Hashimoto & DiBella, 1967) was remarkably

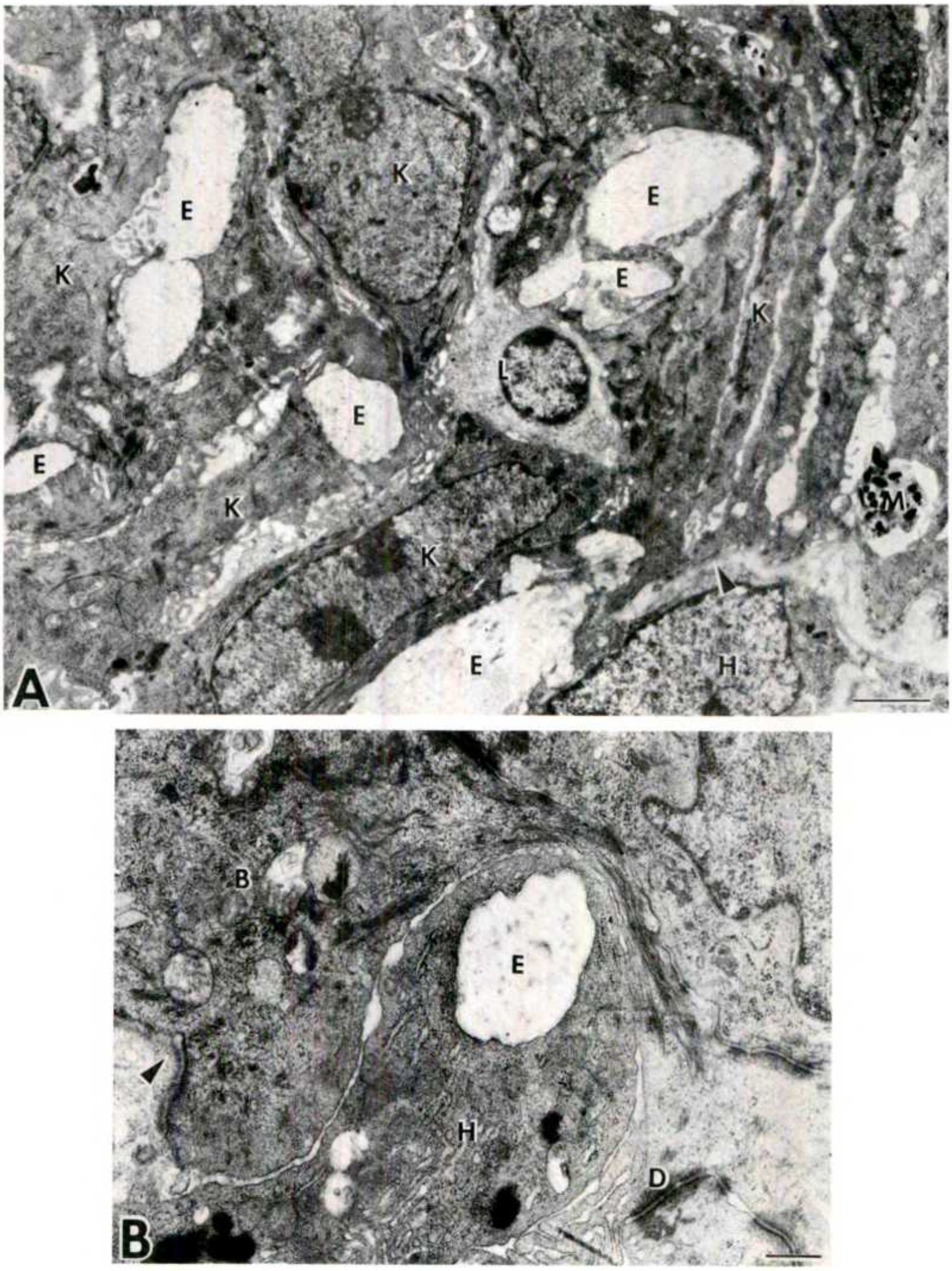

FIGURE 4. A (top). Aggregated elastic fibres (E) accompanied by inflammatory cells penetrate the dermo-epidermal junction. Most keratinocytes (K) are compressed. Arrowhead = basal lamina of epidermis, H = histiocyte, L = lymphocyte. Bar = 1 μm. (× 10,800.) B (bottom). The cytoplasmic process of the histiocyte (H) engulfing an elastic fibre (E) penetrates the basal lamina of epidermis (arrowhead). B = basal keratinocyte, D = desmosome between basal keratinocytes. Bar = 0·5 μm. (× 15,000.)

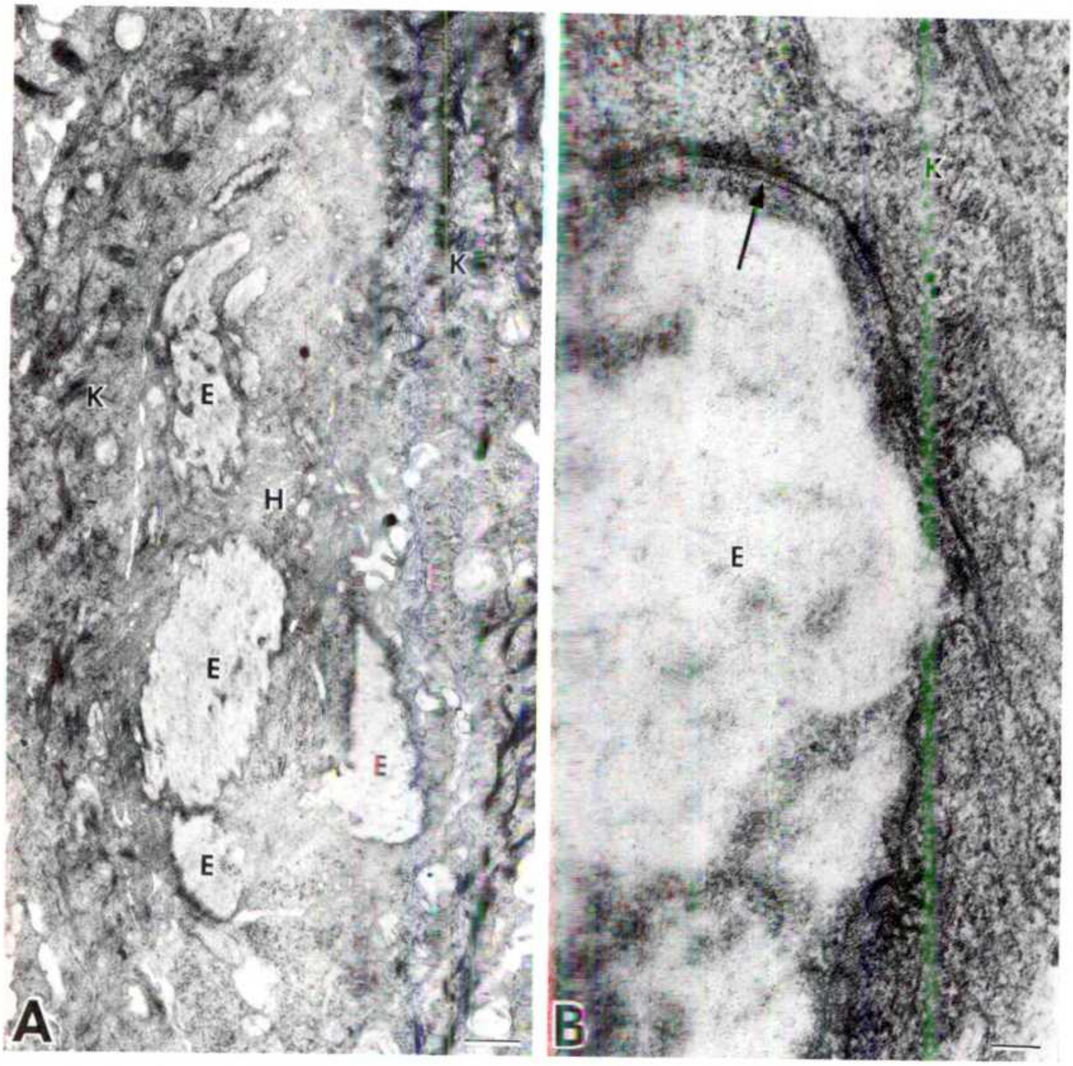

FIGURE 5. A. Elastic fibres (E) enveloped by the cytomembrane of the histiocyte (H) are located in the intercellular space between keratinocytes in the spinous layer of the epidermis. K = keratinocyte. Bar = 1 μm. (× 7400.) Spinous cell (K) forms a hemidesmosome with sub-basal dense layer (arrow) along the surface of an elastic fibre (E). Bar = 0·1 μm. (× 64,000.)

reduced (Fig. 6). These findings were interpreted as indicating that the elastic fibres were shed together with the horny cells from the skin surface.

DISCUSSION

The process of wound healing of the skin is fundamentally the same as in all other tissues. Removal of inflammatory material and necrotic debris is followed by replacement or reconstruction of the original tissue (Boyd, 1970). Contrary to the usual healing process, the

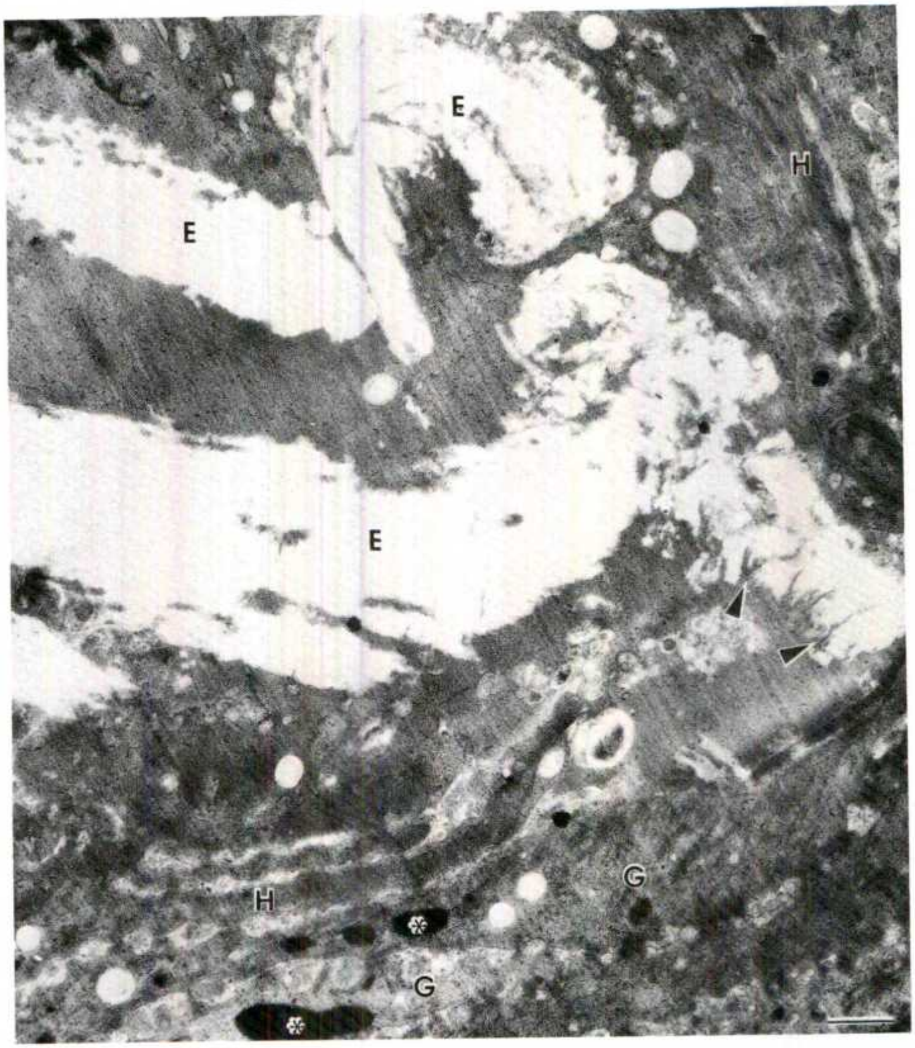

FIGURE 6. There are elastic fibres (E) in the horny layer. They are cleaved transversely in some areas (arrowheads), and the dense granular substance is remarkably reduced in amount. G = granular cell, H = horny cell. Asterisks = keratohyalin granule. Bar = 1 μm. (× 8600.)

present study has demonstrated the interesting phenomenon of transepithelial elimination of elastic fibres even after the epidermis had regenerated. This phenomenon in a healing wound of the skin was originally described by Pinkus (1965).

Generally speaking, transepithelial elimination results from the interaction between an extrinsic or intrinsic foreign body, the dermal component and the epithelial component (Mehregan, 1970). In this case the foreign body is elastic fibre, the dermal component is inflammatory cells, and the epithelial component is the regenerated epidermis.

Both eliminated and non-eliminated elastic fibres had normal histochemical and ultrastructural properties and they were therefore intact morphologically. DIF did not demonstrate immunoglobulins or complement components binding to the surface of eliminated elastic fibres, suggesting that no significant humoral immunological mechanism is involved. Nevertheless, a number of lymphocytes contacted elastic fibres directly and the cytomembrane of the histiocytes enveloped them. These reactions may indicate sufficient alteration of the biochemical properties of these elastic fibres to elicit a foreign body reaction.

Unlike elastosis perforans serpiginosa (Mehregan, 1968) and perforating folliculitis (Mehregan & Coskey, 1968) the epidermis did not show any pseudo-epitheliomatous hyperplasia surrounding the eliminated elastic fibre. Although phagocytic activity of keratinocytes has been described in some other conditions (Malak & Kurban, 1971; Takahashi *et al.*, 1983), the keratinocyte appeared passively compressed by the eliminated elastic fibre and did not exhibit signs of phagocytic activity. These findings may indicate that the regenerated epidermis does not participate actively in the transepithelial elimination of elastic fibre. The formation of hemidesmosomes between keratinocytes and elastic fibres has been observed in various situations (Hashimoto *et al.*, 1973) and seems to be non-specific. Therefore, since maturing keratinocytes produce a stream of cells moving continuously upward (Mehregan, 1970), the regenerated epidermis may function only as a conveyor to carry elastic fibres to the skin surface.

In contrast, inflammatory cells, especially lymphocytes and histiocytes, may play a very important role in the recognition of elastic fibres as foreign body and in the actual removal of these fibres. It is not clear why some elastic fibres are eliminated. It is possible that rapid formation of elastic fibres occurs in the inflammatory foci of wound healing. Defective fibres may be formed and are therefore eliminated. Alternatively, these elastic fibres may represent the remnants of damaged fibres in the deep dermis which are subsequently removed.

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Epidermal T lymphocytes and HLA-DR expression in psoriasis

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SUMMARY

Double staining immunofluorescent techniques and monoclonal antibodies were used to study the numbers, distribution, HLA-DR expression and relationship of T-cell subpopulations and dendritic cells in psoriatic skin. In the dermis there was a definite increase in both T helper and T suppressor cells in uninvolved skin of psoriatic patients, and the appearance of clinical lesions was not associated with any detectable change in the numbers of these cells in the dermis. In contrast, eruption of skin lesions was associated with an increase in the numbers of epidermal HLA-DR$^+$ dendritic cells and also with epidermal influx and activation of T helper cells, while resolution of lesions coincided with increased epidermal entry and activation of T suppressor cells. Both the T helper and T suppressor cells were preferentially found adjacent to epidermal dendritic cells.

These findings suggest that the clinical activity of psoriasis may be dependent upon the interaction of T helper and suppressor cells with antigen-presenting cells in the epidermis.

Several recent reports have implicated immunological mechanisms in the pathogenesis of psoriasis (Obalek, Haftek & Glinski, 1977; Glinski et al., 1978; Sauder *et al.*, 1980; Gladman *et al.*, 1981). It has been demonstrated that the lymphocytic infiltrate in the dermis of both early and chronic psoriatic lesions is largely composed of T cells (Stingl *et al.*, 1977; Bjerke, Krogh & Matre, 1978; Bos *et al.*, 1983), and a comparison of T helper (T_H)/T suppressor (T_S) ratios in the blood and dermis of psoriatic patients made recently by our group (Baker *et al.*, 1984) suggested an active recruitment of T_H cells from the blood into psoriatic lesions. However, very little has so far been reported about mononuclear leukocytes in psoriatic epidermis. Two groups using respectively ATP-ase staining and monoclonal antibodies against HLA-DR antigens have demonstrated clustering of epidermal Langerhans cells (Muller, Winkelman & Vanderstein, 1971; Morhenn, Abel & Mahrle, 1982) whilst more recently the sporadic infiltration of T_S cells into psoriatic epidermis has been reported (Bos *et al.*, 1983).

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It is now known that T_H cells are only activated when they are presented with antigens which are linked to immune response gene-associated (Ia) molecules (HLA-DR in man) on the surface of specialized antigen-presenting cells (Bergholtz & Thorsby, 1977). These cells include Langerhans cells and release interleukin I which is required for further T-cell proliferation and differentiation (Smith *et al.*, 1980). In man HLA-DR molecules are predominantly expressed by B lymphocytes, dendritic macrophages and to a lesser extent by endothelial cells, but not by resting T cells. However, stimulated T cells can synthesize HLA-DR molecules which are therefore a feature of activated T cells (Albrechtsen, Solheim & Thorsby, 1977; Fu *et al.*, 1978).

In this study we have used a double-labelling immunofluorescent technique with monoclonal antibodies to both DR antigens and T-cell subpopulations. The aim was to determine, separately for epidermis and dermis, the numbers, distribution, relationship and DR expression of T-cell subpopulations and Langerhans cells in psoriatic lesions at different stages of development.

METHODS

Patients

Twenty patients with guttate psoriasis were studied including a small number who also had existing chronic plaques. Two further patients who also had guttate psoriasis had serial biopsies over 3 months, and comprised a longitudinal study. Guttate psoriasis was defined as a sudden outbreak of lesions less than 5 mm in diameter in the 8 weeks prior to examination.

None of the patients had been on systemic treatment, including PUVA, for at least 3 months, and all local treatments were stopped at least 2 weeks before biopsy.

Skin biopsies

Guttate psoriasis was subdivided into early (EG) and late (LG) lesions. EG lesions were 2 mm or less in diameter and less than 1 week old, whilst LG lesions were more than 1 cm in diameter and mostly 3–8 weeks old. In some cases both EG and LG lesions were biopsied from the same patient. Uninvolved skin was biopsied at least 1 cm away from affected skin. The 2-mm punch biopsies were immediately frozen in liquid nitrogen, embedded in Tissue Tek II O.C.T. compound (Lamb, London), and stored at −80 °C. Five-μm sections were cut on a cryostat (Bright Instruments Co. Ltd., Huntingdon, England), air-dried for at least 30 min and either stained immediately or stored at −80 °C.

Monoclonal antibodies

T-cell subsets were identified with Leu 2a (suppressor/cytotoxic T cells) and Leu 3a (helper/inducer T cells) antibodies (Becton Dickinson, Sunnyvale, Calif.). Although each subset is functionally heterogeneous they will for convenience be designated as T_H (Leu $3a^+$) and T_S (Leu $2a^+$). OKT6 antibody (Ortho Pharmaceuticals Corporation, Raritan, NJ) was used because in the skin it reacts selectively with Langerhans cells (Murphy *et al.*, 1981; Fithian *et al.*, 1981). A further monoclonal antibody YE2/36 HLK, reactive with a non-polymorphic region of human HLA-DR antigens, was provided by Dr McConnell, Mechanisms in Tumour Immunity Unit, MRC Centre, Cambridge. YE2/36 HLK reacts strongly with all B lymphocytes, 40% of peripheral blood monocytes and with approximately 50–60% of PHA-activated T cells (Brickell *et al.*, 1981). Cells reactive with this antibody will be designated as DR^+.

Fluorescent labelling

After drying at room temperature the skin sections were fixed in a chloroform/acetone mixture (1:1, v/v) for 10 min at 4°C, and washed in phosphate-buffered saline (PBS; pH 7·4). Staining for T-cell subsets and Langerhans cells was carried out using the biotin–avidin–fluorochrome (BAF) technique. Sections were given 20 min incubation with Leu 2a, Leu 3a or OKT6, diluted 1:5, followed by biotin-conjugated goat anti-mouse antibody (Tago, Burlingame, Calif.) diluted 1:50 and finally rhodamine-labelled avidin (Vector Laboratories, Burlingame, Calif.), diluted 1:100. The sections were washed in PBS between each incubation.

Sections were stained for HLA-DR antigens using the indirect immunofluorescent (IIF) technique. After 20 min incubation with anti-HLA-DR the sections were washed in PBS, incubated for a further 20 min with a 1:30 dilution of fluorescein-labelled rabbit anti-rat antibody and finally washed again in PBS. When sections were stained simultaneously for either T_H, T_S or OKT6 and for HLA-DR expression, the BAF technique was immediately followed by IIF on the same section.

Controls consisted of sections in which the first or the second monoclonal antibody was omitted, and sections which were incubated with the fluorochrome conjugates only. All sections were mounted in 10% PBS in glycerol under coverslips sealed with nail varnish, and examined under a Leitz fluorescent microscope (Wetzlar, Germany) equipped with selective filters for fluorescein isothiocyanate (FITC: green) and tetraethylrhodamine isothiocyanate (TRIC: red).

Expression of results and statistical analysis

Dendritic DR^+ cells present in the epidermis of 100 high power fields (×50 objective) were counted. These dendritic cells were situated mainly at the suprabasal level in the epidermis and were most probably Langerhans cells, but as this was not confirmed by electron microscopy they are referred to as DR^+ dendritic cells. Each group of counts was expressed as the mean ± standard error. The difference between the means was analysed using the Student's *t*-test.

The numbers of T cells and OKT6 reactive cells were graded as follows: $(+) = 1$–4, $+ = 5$–9, $++ = 10$–19, $+++ = 20$–99 and $++++ \geqslant 100$ cells per section. When T-cell numbers were sufficient the percentage of HLA-DR-positive T cells was determined for each subset.

RESULTS

Normal and uninvolved psoriatic skin

A mean of 356 ± 30 DR^+ dendritic cells were present in 100 HPF of epidermis of normal skin. The corresponding value for uninvolved skin was 422 ± 36, this value not differing significantly from the mean for normal skin (Fig. 1). In these biopsies the cells were evenly distributed suprabasally with only occasional DR^+ cells in the basal position. T_H cells were hardly ever seen in normal and uninvolved epidermis, whilst small numbers of T_S were present in the basal epidermis of most specimens from uninvolved skin and in less than half of those from normal skin. No epidermal DR^+ T cells were found in either normal or uninvolved skin.

In the dermis of normal skin only a few T cells were seen, consisting of approximately equal numbers of T_H and T_S cells, both cell types being mostly DR^+. In uninvolved dermis however there was a marked increase in both T_H and T_S cells, and nearly all specimens contained DR^+ T_H cells, whilst DR^+ T_S cells were seen in the dermis of only seven out of twenty specimens from uninvolved skins.

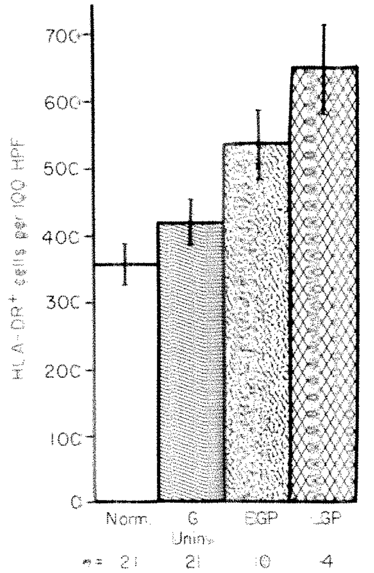

FIGURE 1. Numbers of epidermal DR$^+$ dendritic cells in normal, uninvolved psoriatic and lesional psoriatic skins. Counts expressed as mean ± standard error. n = number of biopsies counted; G Uninv. = guttate uninvolved; EGP = early guttate psoriasis; LGP = late guttate psoriasis.

Lesional psoriatic skin

Epidermis of early guttate lesions. As can be seen in Fig. 1, epidermal DR$^+$ dendritic cells were significantly increased in EG lesions compared with normal skin (542 ± 48, $P < 0{\cdot}01$). Their count was also higher than that of uninvolved skin but the difference was not statistically significant. The distribution of DR$^+$ dendritic cells was similar to that of uninvolved skin except that in most specimens from the early lesions occasional small aggregates were seen. However, in contrast to uninvolved skin a small number of T_H cells were seen in the basal epidermal layer of seven out of the twelve biopsies from EG lesions (Fig. 2). Moreover, some of these T_H cells were

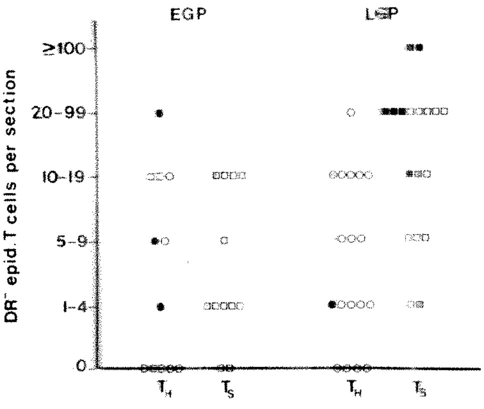

FIGURE 2. Number and HLA-DR expression of T-cell subsets in the epidermis of biopsies of psoriatic lesions. (○) DR$^-$ T_H in biopsy; (□) DR$^-$ T_S in biopsy; (●) DR$^-$ and DR$^+$ T_H in biopsy; (■) DR$^-$ and DR$^+$ T_S in biopsy. (Numbers of DR$^+$ T cells not given.)

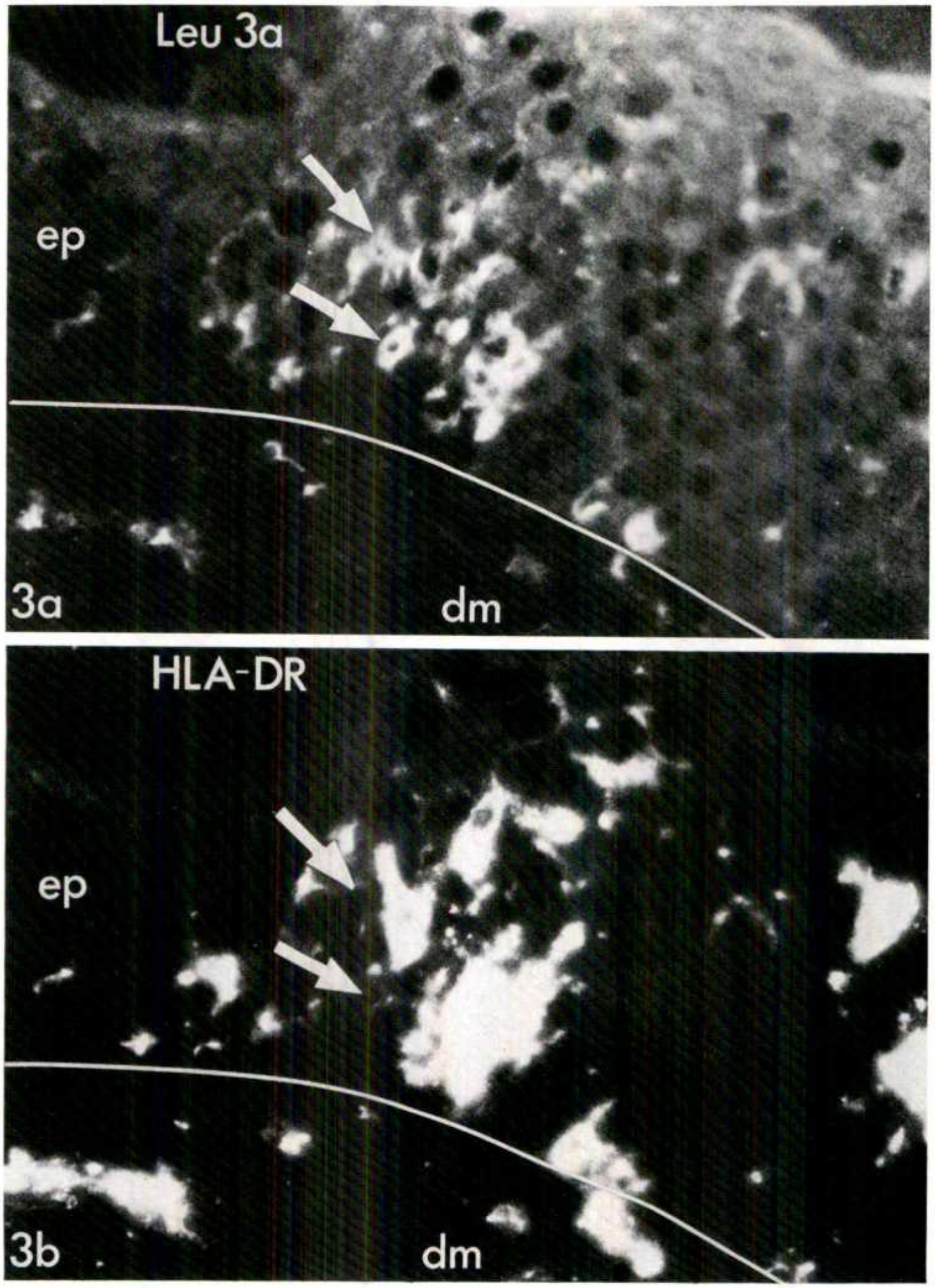

FIGURE 3a, b. Cryostat section of an EG lesion stained with (a) Leu 3a (rhodamine) and (b) anti-HLA-DR (fluorescein) antibody combination. Small numbers of T_H (Leu 3a$^+$) cells (→) in the basal layer of the epidermis are closely associated with DR$^+$ dendritic cells. ep = epidermis; dm = dermis.

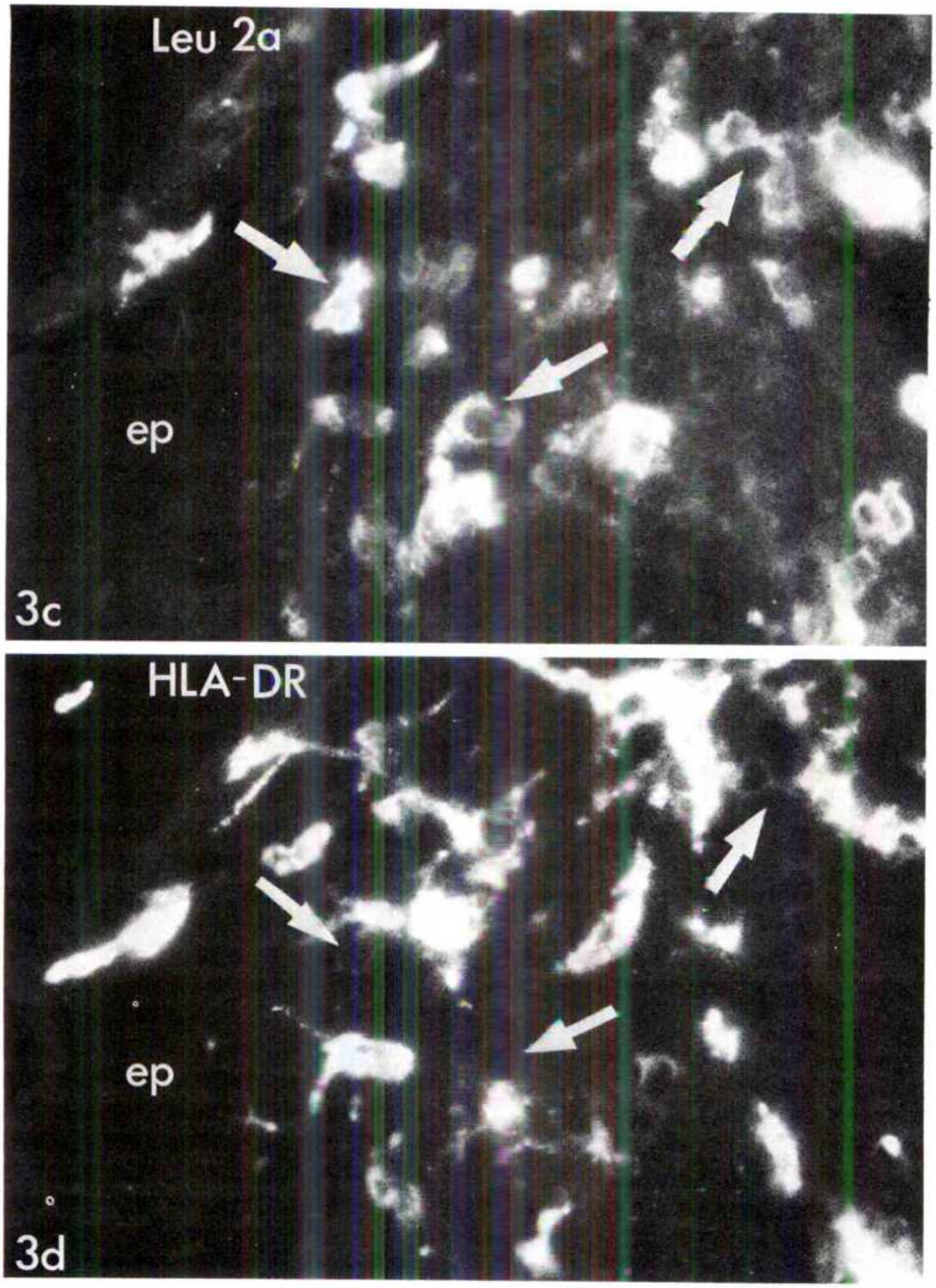

FIGURE 3c, d. Cryostat section of an LG lesion stained with (c) Leu 2a (rhodamine) and (d) anti-HLA-DR (fluorescein) antibody combination. Several T_s (Leu 2a$^+$) cells (→) are scattered throughout the epidermis, some of which are in close apposition to DR$^+$ dendritic cells. ep = epidermis; dm = dermis.

DR$^+$ and they were usually observed in close proximity to DR$^+$ dendritic cells (Fig. 3). The number of T_S cells in EG epidermis was similar to that in uninvolved epidermis (T_H/T_S ratio of approximately 1·0).

Epidermis of late guttate lesions. Epidermal DR$^+$ dendritic cells were significantly increased in LG lesions (656 ± 66) compared with both normal ($P < 0.001$) and uninvolved ($P < 0.01$) skin, but not compared with EG lesions (Fig. 1). A marked clumping of DR$^+$ dendritic cells was observed in the upper epidermis whilst only single DR$^+$ cells were scattered in the rete ridges.

Overall there was a marked increase in the number of epidermal T_S cells in the LG lesions (Fig. 2), and moreover, DR$^+$ T_S cells were seen in six out of eighteen specimens from these lesions. However, in contrast to EG lesions, no DR$^+$ T_H cells were seen although the number of T_H cells in LG lesions was similar to that in EG epidermis, giving a T_H/T_S ratio of approximately 0·50 (Fig. 2). In these late lesions both T_H cells and, more frequently, T_S cells were seen in close apposition to epidermal DR$^+$ dendritic cells (Fig. 3).

Longitudinal studies. Figure 4 shows the numbers of T_H and T_S cells in the epidermis of sequential biopsies taken from lesions in a single patient between 4 and 15 weeks after the onset of guttate psoriasis. There was a progressive decline in the number of T_H cells with a corresponding increase in T_S cells. Moreover, at 12 weeks a significant number of DR$^+$ T_S cells appeared and this coincided with resolution of the lesions. A similar pattern was observed in a sequential study of another patient (data not shown).

Phenotypic heterogeneity of dendritic cells

In normal and uninvolved psoriatic skins only 50% of the total number of OKT6$^+$ dendritic cells were also DR$^+$, as shown by double-labelling techniques. In contrast, in lesional psoriatic skin not only was the total number of dendritic cells increased, but approximately 80% of these cells were DR positive. Furthermore a subset of DR$^+$ dendritic cells did not express OKT6 antigen; such cells were not observed in normal and uninvolved psoriatic skins.

The expression of DR and/or OKT6 antigens by keratinocytes was also occasionally observed in the epidermis of both normal and psoriatic (lesional and uninvolved) skins.

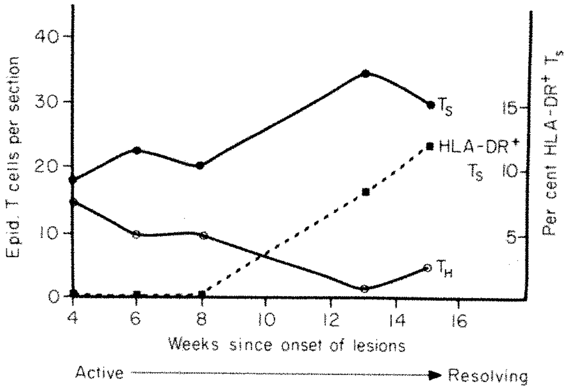

FIGURE 4. Numbers of T_H and T_S cells in the epidermis of sequential biopsies taken from lesions in a single patient with guttate psoriasis.

TABLE 1. T lymphocyte subpopulations and HLA-DR expression in the dermis of uninvolved and lesional psoriatic skin

Skin	Total T_H	DR$^+$ T_H	Total T_S	DR$^+$ T_S	DR$^+$/OKT6$^+$
Guttate uninvolved ($n = 10$)	+ + +	42%	+ +	(+)	+
Early guttate ($n = 12$)	+ + +	39%	+ +	(+)	+
Late guttate ($n = 18$)	+ + + +	40%	+ + +	(+)	+ +

The mean number of cells present per section are expressed with the following symbols: (+) = <5, + = 5–9, + + = 10–19, + + + = 20–99, + + + + = ≥100. Proportions of DR$^+$ T cells are expressed as percentages only where sufficient numbers were present. n = number of biopsies.

Dermis

The dermis of EG lesions showed an increase of T_H and T_S cells compared with normal skin, but the increase was the same as uninvolved skin. LG dermis however, showed a further increase in T-cell infiltration involving both T_H and T_S cells (Table 1), with the great majority of DR$^+$ T cells in both EG and LG dermis being of the T_H subset. DR$^+$/OKT6$^+$ cells were also increased in LG, but not in EG and uninvolved dermis. These cells were mainly observed in T-cell aggregates, but were also occasionally found near the dermal/epidermal junction and scattered in the dermal papillae.

DISCUSSION

This study shows that there is an abnormal infiltration of both T_H and T_S cells in uninvolved dermis of psoriatic patients and that the appearance of clinical lesions is not characterized by any detectable change in the number of DR expression of these cells in the dermis. In contrast, two striking epidermal changes are associated with the eruption of psoriatic lesions. Firstly, an increased number and small aggregates of DR$^+$/OKT6$^+$ dendritic cells and secondly, epidermal influx of T_H cells, some of which are activated as judged by DR expression. In these early lesions the T_H cells are usually in close contact with the dendritic cells and situated in the basal layer of the epidermis. It should be noted that only a few scattered T_S cells but no T_H cells can be detected in uninvolved psoriatic epidermis.

In contrast to our findings, a recent study by Haftek *et al.* (1983) demonstrated reduced numbers of OKT6$^+$ and DR$^+$ dendritic cells in involved psoriatic epidermis. However, their values were expressed as the mean number per epidermal section surface unit in order to obtain a ratio between DR$^+$ (Langerhans cells) and DR$^-$ (keratinocytes) epidermal cells. Our counts, on the other hand, are expressed as the number per length of epidermis present in 100 high power fields to give the absolute numbers of DR$^+$ dendritic cells in psoriatic epidermis. Although the presence of DR$^+$ keratinocytes could account for the raised DR$^+$ dendritic cell numbers reported here, the marked difference in staining pattern of the two cell types makes this unlikely. Furthermore, variations in the numbers of epidermal DR$^+$ dendritic cells as defined by different monoclonal antibodies have been recently reported (Harrist *et al.*, 1983).

Our findings therefore suggest that the entry of T_H cells into the epidermis and their subsequent activation and interaction with antigen-presenting epidermal cells may be required for the initiation of psoriatic lesions. This interaction may in turn lead to the production of a variety of biological activities including one which may stimulate epidermal growth (Korszun, Wilton & Johnson, 1981).

On the other hand, T_S cells are usually more prominent than T_H cells in the epidermis of the late guttate lesions. Furthermore, in contrast to the early lesions, DR^+ T_S cells were present in specimens from six out of eighteen late lesions, some of which had started to fade when the biopsy was taken. This trend was confirmed by a study of sequential skin biopsies from a single psoriatic patient in whom fading of the lesions coincided with increasing numbers and the appearance of DR^+ T_S cells. It is therefore conceivable that the resolution of psoriasis lesions is dependent upon epidermal recruitment and activation of T_S cells.

The mechanisms by which T_S cells suppress immune reactions have not yet been fully elucidated. However, Ptak and Gershon (1982) have demonstrated that, in mice, antigen-presenting cells can be targets of T_S cells. They showed that factors released by antigen-specific T_S are able to combine with antigen on antigen-presenting cells, rendering them incapable of activating either the helper or suppressor systems of contact sensitivity responses. As T_H cells can recognize antigen only when it is presented in conjunction with DR antigens on macrophages, an alternative mechanism of suppression could be the blocking or removal of the HLA-DR antigen. Studies to investigate the latter possibility are currently in progress at this laboratory.

It is in any case clear that a consistent relationship exists between different stages of development of psoriatic lesions and the numbers and activity of different T-cell subpopulations and Langerhans cells in the epidermis. In contrast there is no obvious relationship between clinical features and the nature of the cellular infiltrate in the dermis. It should be pointed out that in the present study T cells were counted in the epidermis of only one section from each lesion. The numbers observed are therefore rather low and although this does not significantly affect the overall trend it could explain an apparent lack of epidermal T_H cells in some of the EG lesions and the absence of epidermal DR^+ T_S cells from many of the LG lesions.

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Decreased natural killer cell activity in generalized pustular psoriasis (von Zumbusch type)

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SUMMARY

Mononuclear cells isolated from the peripheral blood of four untreated patients with pustular psoriasis (von Zumbusch type) displayed greatly diminished natural killer cell activity against K-562 erythroleukaemic target cells in a ^{51}Cr release assay when compared with mononuclear cells from healthy controls.

Pustular psoriasis of the von Zumbusch type is a rare variety of psoriasis with generalized superficial pustules (Baker & Ryan, 1968). Deposition of immune complexes in the stratum corneum, activation of complement (Beutner *et al.*, 1975; Jablonska *et al.*, 1979) and release of chemotactic C5a complement component (Lazarus, Yost & Thomas, 1977) might be responsible for chemoattraction of polymorphonuclear leukocytes (PMN) into the epidermis (Tagami & Ofuji, 1982). PMN chemotaxis was found to be significantly increased in active psoriasis compared with controls (Christophers, Schroeder & Langner, 1982), and reached extreme values in pustular psoriasis (Langner *et al.* 1983). Beutner (1982) has reviewed the role of PMN and immunological mechanisms in psoriasis.

One of the important defence mechanisms of an organism is natural killer cell (NKC) activity (Roder & Pross, 1982; Herberman, 1982; Goldfarb & Herberman, 1982). NK cells are considered to play an important role in host defence against neoplastic cells, viruses, microbial infections and bone marrow transplants as well as in graft-versus-host disease, and in the regulation of haematopoiesis (Herberman, 1982). Abnormal NK cell activity has been found in some systemic disorders such as in systemic lupus erythematosus (Tsokos *et al.*, 1982; Katz *et al.*, 1982) and systemic sclerosis (Wright, Hughes & Rowell, 1982).

In preliminary studies we found a diminished NKC activity in patients with psoriasis vulgaris (Kamiński *et al.*, 1983). The present investigation was planned to test the NKC activity of

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mononuclear cells isolated from peripheral blood of patients with pustular psoriasis (von Zumbusch type).

METHODS

Four female patients suffering from generalized active pustular psoriasis were examined. They had not received any systemic therapy at the time of study. The clinical data are shown in Table 1. The extent of the lesions was evaluated as a percentage of the skin involved in various body areas. The activity of the disease was graded as: +, sparse pustules on an inflamed base, appearing in small crops; + +, numerous new pustules appearing in a particular area; + + +, very abundant pustules continually appearing and/or epidermal necrolysis.

Ten healthy individuals and ten untreated patients with psoriasis vulgaris were studied for comparison. As an additional control, four patients with active SLE were included in the study, since NKC activity is diminished in the active disease (Katz *et al.*, 1982; Tsokos *et al.*, 1982; Kozlowski, Hirsen & Jackson, 1982).

Mononuclear cell isolation. Ten to fifteen ml samples of heparinized blood were diluted with phosphate-buffered saline (PBS) (1 : 1), layered on Ficoll–Uropoline gradient and centrifuged to obtain a mononuclear cell suspension (Kaminski *et al.*, 1981). The isolated mononuclear cells were rinsed three times with PBS, counted and resuspended in 20% fetal calf serum (FCS) in TC 199 medium supplemented with gentamicin, L-glutamine and Hepes buffer.

Measurement of NK cell activity. A K-562 erythroleukaemic cell line was used as the target for the estimation of NK cell activity. The target cells were propagated at standard conditions in 10% FCS in RPMI 1640 supplemented with gentamicin and L-glutamine. Before use, target cells were labelled with ^{51}Cr for 1 hour at 37°C, rinsed and added to 1 ml ampoules in aliquots of 2×10^4 cells in 50 μl of medium (20% FCS in TC 199 with gentamicin, L-glutamine and Hepes buffer). Blood mononuclear cells were then added to the ampoules to obtain an effector : target cell ratio of 30 : 1 in a total volume of 250 μl of medium. All tests were repeated 6 times. The ampoules were then incubated for 18 h at 37°C in a humidified atmosphere with 5% CO_2. After incubation the ampoules were centrifuged, and 100 μl samples of supernatant were counted in a gamma counter (Beckman). Spontaneous release was estimated by incubation of target cells without effector cells, and maximal release was evaluated by adding 0·5% Triton X-100 to target cells. The percentage of specific lysis was calculated according to the formula:

$$\frac{\text{test c.p.m.} - \text{spontaneous c.p.m.}}{\text{maximal c.p.m.} - \text{spontaneous c.p.m.}} \times 100$$

RESULTS

The results are shown in the Table. Mononuclear cells isolated from the peripheral blood of patients with pustular psoriasis showed a much reduced natural killer cell cytotoxicity, comparable to that seen in patients with active SLE. Cells from patients with psoriasis vulgaris showed diminished cytotoxicity compared with healthy controls. However, the mean cytotoxicity in this group was significantly higher than in patients with pustular psoriasis. Variations in NKC activity expressed as variation coefficients (vc) (Freund, 1969) were lowest in the pustular psoriasis group, (vc = 19·61), intermediate in the healthy control group (vc = 36·21), and highest in psoriasis vulgaris (vc = 80·08).

TABLE 1. Natural killer cell cytotoxicity of peripheral blood mononuclear cells in patients with generalized pustular psoriasis

Patient	Age	Duration of the disease (years)	Duration of the last relapse	Extent of the lesions (%)	Activity of the disease*	NKC activity**
1	53	2	1·5 months	90	+ +/+ + +	16·9 ± 1·2
2	76	26	4 days	50	+/+ +	13·3 ± 1·6
3	51	20	4 days	90	+ + +	21·7 ± 2·0
4	34	8	7 days	25	+/+ +	17·2 ± 3·2
						Mean 17·3 ± 1·7 ($n = 4$)
Healthy controls						Mean 54·4 ± 6·2 ($n = 10$)
Psoriasis vulgaris						Mean 30·0 ± 7·6 ($n = 10$)
SLE patients						Mean 16·3 ± 3·6 ($n = 4$)

* See text for grading of activity.

** NKC activity expressed as mean values ± s.e. of percentage of specific ^{51}Cr lysis in 18 h assay at effector/target ratio 30:1.

Pustular psoriasis and SLE groups differ from all the others (Student's t-test, $P < 0·01$).

DISCUSSION

We found that the natural killer cell activity was reduced in patients with pustular psoriasis of von Zumbusch type. NK cell activity was decreased to a lesser degree in psoriasis vulgaris patients, although here also it was significantly lower than in healthy controls. In SLE patients the NKC activity was found to be of the same order as in pustular psoriasis. The mechanism of the decrease in NKC activity in SLE depends on the presence of cytotoxic antibodies to NK cells (Rook *et al.*, 1982). In pustular psoriasis, other factors seem to be responsible for the diminished activity.

A conspicuous histopathological feature of pustular psoriasis is a massive epidermal infiltration by polymorphonuclear cells and intense microabscess formation (Pinkus & Mehregan, 1966). Activation of polymorphonuclear cells is thought to be an important event in the pathogenesis of the disease (Jablonska *et al.*, 1979; Sedgwik, Bergstresser & Hurd, 1981; Bergstresser, Sedgwick & Hurd, 1982; Gliński *et al.*, 1982). Recently it was shown that polymorphonuclear cells may inhibit *in vitro* NK cell activity (Kay & Smith, 1983). Such an inhibitory effect of PMNs, if it occurs also *in vivo*, might be responsible for the observed decrease in NK cell activity. Since the involvement of polymorphonuclear cells in pustular psoriasis reaches an extreme level, NK cell activity is more severely affected than in common psoriasis. However, the NK cell activity is not completely abolished, probably due to other regulatory mechanisms which do not depend on polymorphonuclears.

It remains to be seen whether the decreased NKC activity is important in the pathogenesis of pustular psoriasis, and whether it occurs to some extent in all neutrophil-mediated diseases.

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Culture of normal adult human melanocytes*

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SUMMARY

Melanocytes isolated from normal adult human skin were cultured *in vitro*. Separation of the epidermis from the dermis by trypsin flotation proved better than collagenase treatment for providing viable cultures of melanocytes with a minimum of fibroblast contamination. Centrifugation on a discontinuous, rather than a continuous Percoll gradient, was more efficient in separating the epidermal cell types. Most of the melanocytes were usually found in one particular layer, and most of the viable keratinocytes were in the sediment. None of the layers produced a uniformly high percentage of melanocytes on routine culture, but enriched melanocyte cultures could be obtained by seeding the epidermal cells in magnesium- and calcium-free medium for 24 to 48 hours, and then transferring them to fibroblast-conditioned medium containing horse serum and polyamines. Melanocytes were identified by their dendritic morphology, ultrastructure, reaction to cholera toxin and pigment production after treatment with melanocyte stimulating hormone. Pure cultures of melanocytes have been cultivated by this method for more than 43 weeks (ten passages).

Pigment cells are widely used for studies in cell biology (Bagnara & Hadley, 1973; Seiji, 1981; Nielsen, 1983). Most of the work has been done on the dermal melanophore of lower vertebrates (Nielsen, 1978, 1980; Nielsen & Dyck, 1978; Nielsen & Bereiter-Hahn, 1982), and its mammalian epidermal counterpart, the melanocyte.

In 1960 Cruickshank and co-workers first grew normal adult human melanocytes *in vitro* (Cruickshank, Cooper & Hopper, 1960) but it has been difficult to obtain sufficiently pure cultures to produce a substantial amount of cells. The problems in cultivating melanocytes *in vitro* are threefold: (1) separation of epidermal cells; (2) purification of the melanocyte culture; and (3) promotion of sustained growth of the melanocytes.

The first step in the isolation of melanocytes is to separate the epidermis, thus reducing the risk of fibroblast contamination. This is usually done enzymatically with collagenase (Hentzer &

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Kobayasi, 1978) or trypsin (Wilkins & Szabo, 1981; Briggaman *et al.*, 1967). A single-cell suspension is then produced enzymatically with trypsin and EDTA, DTE or DTT (Hentzer & Kobayasi, 1976, 1978) or enzymatically in combination with scraping and pipetting (Eisinger & Marko, 1982; Kitano & Harta, 1972).

The next step is purification of the melanocyte culture in order to avoid overgrowth by keratinocytes. Fritsch *et al.* (1979a,b) reported that keratinocytes, unlike melanocytes, required a serum protein factor (present in fetal calf serum) for attachment. Fritsch *et al.* (1979a) also found that keratinocytes attached only in the presence of Mg^{2+} ions, whereas this was not necessary for melanocytes. Wilkins and Szabo (1981) produced cultures of melanocytes with an estimated purity of 95% using high levels of mycostatin that inhibited keratinocyte adhesion. Riley (1975) found that a high oxygen concentration in the culture medium preferentially killed keratinocytes and recently Tsuji and Karasek (1983) observed that methotrexate was differentially toxic for human keratinocytes. Eisinger and Marko (1982) reported that 10 ng/ml of phorbol-12-myristate-13-acetate (PMA) had a toxic effect on human foreskin keratinocytes, and that 10 nM cholera toxin inhibited fibroblast growth. In addition they showed that PMA promoted the sustained growth of foreskin melanocytes (also Eisinger, Marko & Weinstein, 1983).

This paper describes experiments designed to obtain pure proliferating cultures of normal, adult human melanocytes.

METHODS

Human skin was obtained from the thigh or breast of patients undergoing plastic surgery. It was split with a Mandal dermatome either at surgery or in the laboratory after removal of fatty tissue. The thickness of the split skin was around 0·3 mm.

Two means of separating the epidermis from the dermis were used. Following the method of Kobayasi *et al.* (personal communication) skin samples were incubated in collagenase (Worthington, CLS) (about 1400 units per mg skin) for 1–2 hours at 37°C under vigorous shaking. The epidermis was then peeled off the dermis, which was discarded. After incubation in dithioerythritol (DTE, 3 mg/ml) for 7 minutes at 37°C, the epidermis samples were transferred to a trypsin solution (Gibco, 0·2%) and vigorously shaken for 2–3 min. This produced a suspension of single cells which after being washed two to three times with culture medium were ready for further processing.

The second method consisted of floating dermatomed skin on trypsin (Difco, 0·25%) overnight at 4°C (Szabo, 1955) or for 1 hour at 37°C. Dermis and epidermis were then easily separated. Epidermal cells were gently scraped off the stratum corneum with a scalpel and dissociated into a single cell suspension by pipetting. Basal epidermal cells still loosely attached to the basal lamina were shaken off and added to the epidermal cell suspension or cultured separately (Liu & Karasek, 1978).

Attempts were then made to selectively separate the epidermal cell types. The first approach was differential centrifugation on a preformed, continuous gradient of Percoll (Pharmacia) following the method of Kobayasi *et al* (personal communication). The gradient was generated by centrifugation of a 40% Percoll solution in Hank's balanced salt solution at 30,000 ***g*** for 20 min. The single cell suspension was layered on top of the gradient and spun at 800 ***g*** for 25 minutes. Cells from the distinct layers obtained were set up in culture.

In accordance with the results of this method, an attempt was made to improve the separation by using a discontinuous Percoll gradient with densities of 1·050 and 1·065 produced by prior dilution of Percoll with Hank's solution.

A third method of separating epidermal cells was an attempt to selectively inhibit growth of keratinocytes by seeding non-separated single cell suspensions in culture medium that did not contain Mg^{2+} and Ca^{2+}.

The culture medium used was FIB41B (Kieler *et al.*, 1971)—a modification of Eagle's minimum essential medium (MEM) with a twofold concentration of the essential amino acids, a fourfold concentration of the vitamins and glutamine and addition of non-essential amino acids and 10% fetal calf serum.

Initially, the medium was custom-made FIB41B, Mg^{2+}- and Ca^{2+}-free, with fetal calf serum depleted of these ions by the chelation method of Swierenga and MacManus (1982). After 24–48 hours the medium was changed to regular FIB41B and regular fetal calf serum. For the most recent experiments DME was used instead of FIB41B—this apparently made no difference.

The regular FIB41B was sometimes modified by conditioning with monolayers of adult human skin fibroblasts for 24–48 hours (Melbye & Karasek, 1973; Yuspa *et al.*, 1981), and Millipore (0·45 μm) filtering before use. This was similarly done with medium where calf serum had been replaced by 15% horse serum and to which polyamines had been added (4 μM spermidine, 4 μM spermine and 1 μM putrescine—hereafter abbreviated as HSSP (Roszell, Douglas & Irving, 1977; Heby, 1981). Penicillin (250 i.u./ml) and streptomycin (30 μg/ml) were also added to the medium.

Melanocytes were identified by electron microscopy (Philips EM301), by their reaction to cholera toxin (Sigma, C-3012, 10^{-8} M) and occasionally by their pigment production under the influence of melanocyte stimulating hormone (to be published separately).

RESULTS

Adult human skin samples were treated with trypsin or collagenase in order to separate the epidermis from the dermis. Trypsin flotation caused a separation of the skin above the basal lamina (Fig. 1) and basal epidermal cells were often still attached to the basal lamina after separation. These cells could be shaken off, and they produced especially enriched melanocyte cultures. Collagenase attacked the collagen fibrils of the dermis and the basal lamina, whereas the epidermis remained intact due to unbroken desmosomes between the cells (Fig. 2). Trypsin flotation, furthermore, appeared to yield more viable cells than collagenase treatment as judged by growth rate. There was no difference whether skin samples were floated overnight at 4°C or for 1 hour at 37°C.

Percoll gradients were used to selectively separate the epidermal cell types and aid in the establishment of melanocyte cultures.

Single-cell suspensions (10^6–10^8 cells) centrifuged on continuous Percoll gradients produced two layers of cells at densities of approximately 1·10 and 1·05. Cells from each layer were cultivated for 5 to 10 days in regular FIB41B medium plus fetal calf serum, and the percentage of melanocytes found among settled cells was estimated by simple counting. The majority of the melanocytes were found in the 1·05 density layer. There was a marked contamination with keratinocytes due to insufficient separation and difficulties in removing cells from one layer on the gradient without affecting the other.

The latter was overcome by the use of a discontinuous gradient of 1·050 g/ml and 1·065 g/ml density which allowed a greater distance between the layers. This method produced a sediment (layer 3) and two layers of cells that floated on the two respective densities (layer 1 and 2) (Fig. 3). By cell counting it was found that the total recovery of cells after centrifugation (i.e. the sum of all three layers) was 95·8 ± 15·4% of the initial amount of cells put on the gradient

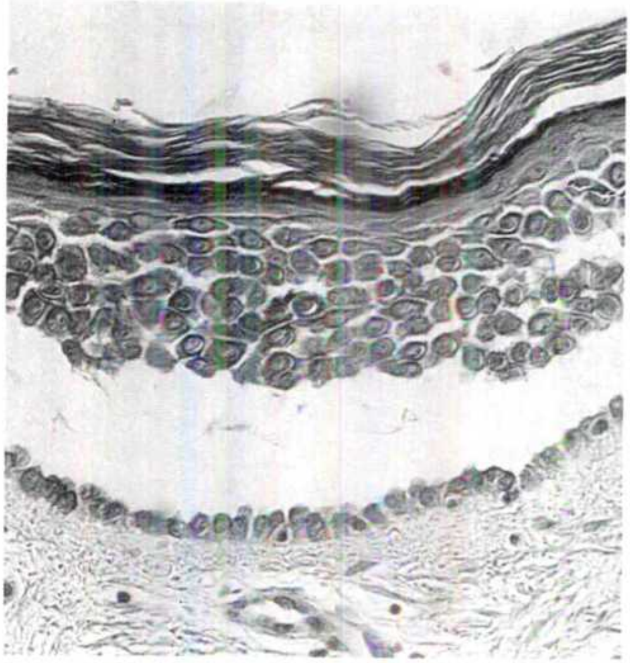

FIGURE 1. Separation of adult human skin by trypsin flotation. Notice the partly dissociated epidermal cells and the layer of basal epidermal cells attached to the intact dermis (× 360).

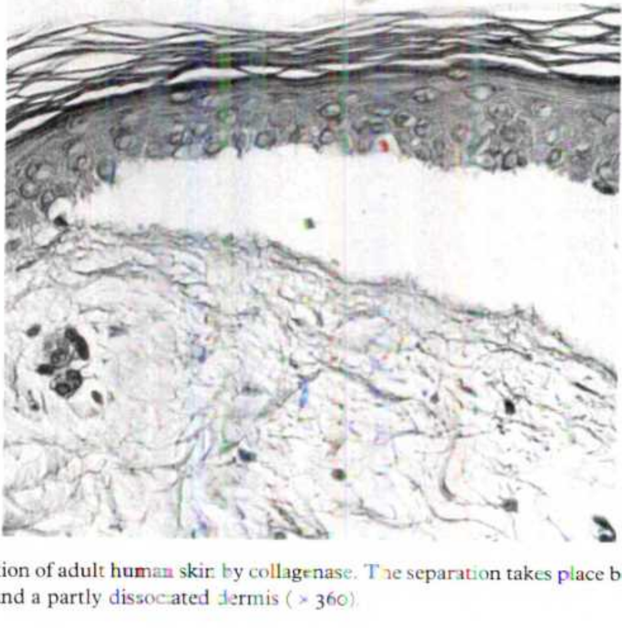

FIGURE 2. Separation of adult human skin by collagenase. The separation takes place between a relatively intact epidermis and a partly dissociated dermis (× 360).

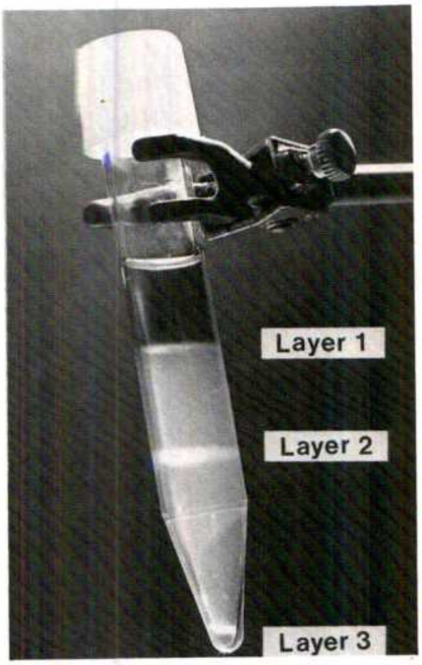

FIGURE 3. Separation on a discontinuous Percoll gradient of a single-cell suspension of human epidermal cells.

(approaching 100% for the latest experiments). From Table 1 it is evident from the cells recovered that the percentage distribution in each layer varied considerably.

When cells from each layer were cultivated for 5–10 days in regular FIB41B medium and fetal calf serum, it was apparent (Table 2) that the variation was very high and that none of the layers uniformly produced cultures with a high percentage of melanocytes. In an attempt to selectively promote the growth of the melanocytes and thereby increase the purity of the cultures, cells were grown in fibroblast conditioned medium that contained either fetal calf serum or HSSP. It was observed (Table 2) that fibroblast conditioning of regular medium enhanced the growth of melanocytes, but also stimulated the growth of keratinocytes (unpublished results). On the other hand, conditioning the medium in the presence of HSSP significantly increased the percentage of melanocytes. This is shown in Table 3, in which the results from all three layers in

TABLE 1. Percentage of recovered cells on each Percoll layer

Layer	Density of underlying Percoll g/ml	% of total cell recovery (mean ± s.d.)	Number of experiments *n*
1	1·050	31·8 ± 36·8	21
2	1·065	10·9 ± 9·0	21
3	(Sediment)	57·3 ± 40·2	21

TABLE 2. Percentage of melanocytes of total number of settled cells after 5–10 days in culture following Percoll gradient centrifugation

Medium	Layer	% Melanocytes (mean ± s.d.)	Number of experiments
A. Regular FIB41B	1	45·9 ± 35·9	11
	2	41·2 ± 38·2	9
	3	32·4 ± 43·2	8
B. Fibroblast-conditioned FIB41B	1	39·3 ± 23·9	10
	2	53·2 ± 33·6	10
	3	32·3 ± 40·4	11
C. Fibroblast-conditioned HSSP-supplemented FIB41B	1	82·6 ± 36·8	7
	2	100·0 ± 0	2
	3	95·3 ± 6·0	6

the gradient centrifugation experiments (Table 2) are pooled. Table 3 shows the percentage of enriched primary cultures of melanocytes (more than 75% of settled cells after 5–10 days in culture). HSSP-supplemented medium appeared to be beneficial in the establishment of cultures that were dominated by melanocytes.

In order to simplify the isolation process an attempt was made to selectively isolate melanocytes by a differential adhesion technique. Single-cell suspensions were seeded in FIB41B medium with low Mg^{2+} and Ca^{2+} content without prior Percoll separation and after 24–48 hours cultured in regular medium or, still better, in fibroblast-conditioned medium containing HSSP. A somewhat higher percentage of enriched melanocyte cultures could be obtained with this technique (Table 3), which also seemed to give a higher yield of viable cells and a greater probability of successful establishment of cell cultures.

Melanocytes isolated by either technique could be subcultured with trypsin (Difco, 0·25%) solution and grown in regular FIB41B (or DME) and fetal calf serum without prior conditioning or modification. Any contaminating keratinocytes were lost upon subculturing.

We have cultured human melanocytes of normal origin for 43 weeks (ten passages) and have been able to freeze and rethaw them.

A few days after seeding, primary cultures of melanocytes had dendritic cells (Fig. 4) that

TABLE 3. Enriched primary cultures with more than 75% melanocytes after 5–10 days. A, B and C are the pooled results of cells separated on a Percoll gradient

Medium	Percentage of cultures
A. Regular FIB41B	14
B. Fibroblast-conditioned FIB41B	12
C. Fibroblast-conditioned, HSSP-supplemented FIB41B	78
D. FIB41B, Mg^{2+}, Ca^{2+} free (no gradient centrifugation)	83

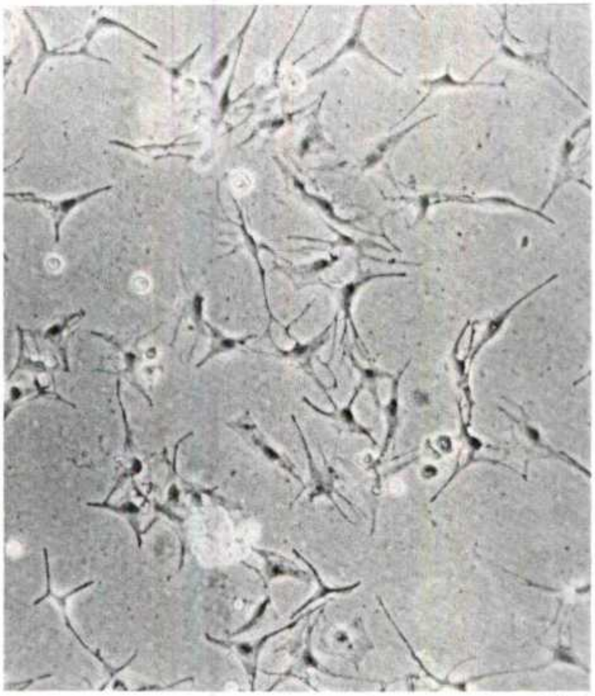

FIGURE 4. Young culture of adult human melanocytes (8 days old) (× 140).

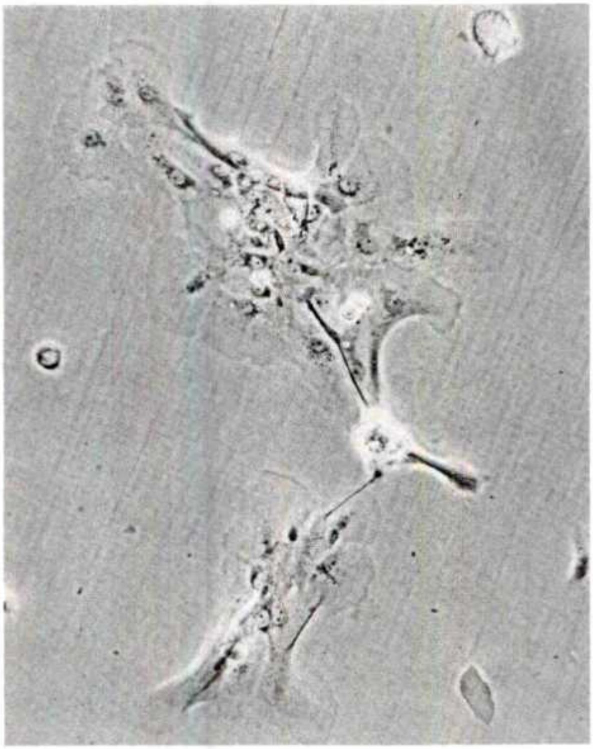

FIGURE 5. Young culture of adult human keratinocytes (7 days old) (× 140).

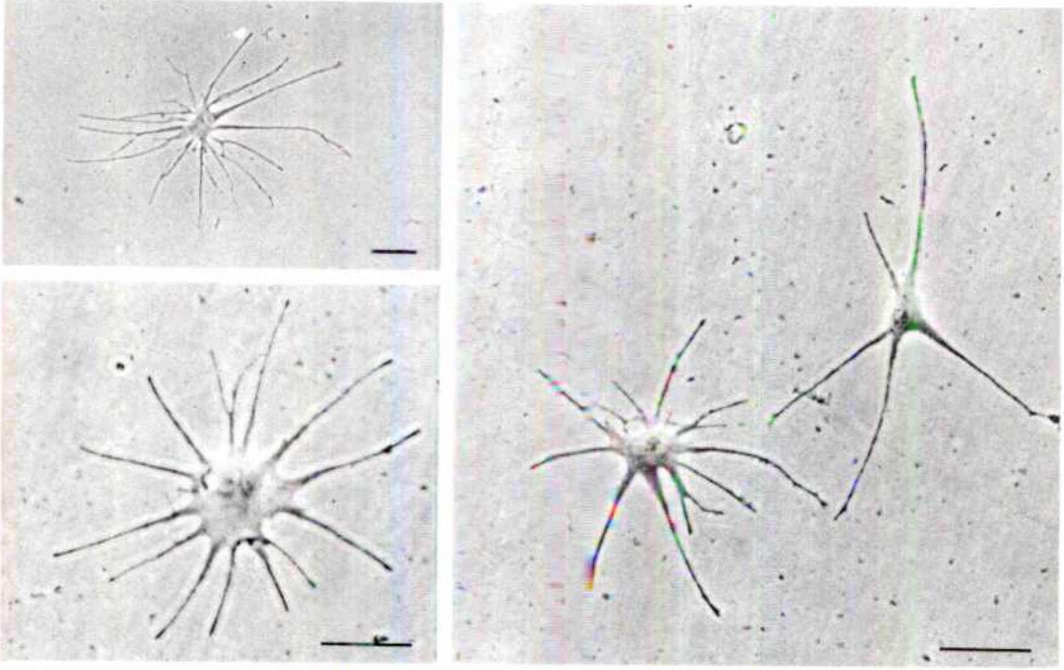

FIGURE 6. Solitary human melanocytes (scale bars indicate 50 μm).

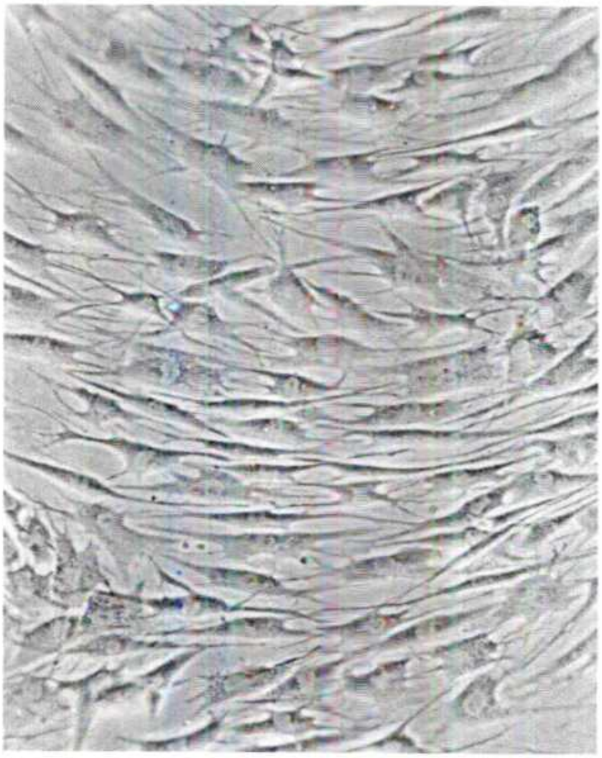

FIGURE 7. Dense growth of adult human melanocytes after 25 days in culture (× 140).

were easily distinguished from keratinocytes (Fig. 5). Some of the solitary melanocytes were highly dendritic (Fig. 6). During cultivation as the cell density increased, the dendritic morphology decreased (Fig. 7), and finally in very dense cultures the cells took on a fibroblastoid appearance. Addition of cholera toxin (10^{-8} M) appeared to increase the growth and dendritic morphology of the melanocytes.

The melanocytes in culture were identified electron microscopically by the presence of premelanosomes in some of the cells and a similar overall ultrastructural appearance of others (Fig. 8). In a few cases treatment of the cultures for 3 weeks with 10^{-7} M α-MSH or (4-Nle, 7-D-Phe)-α-MSH (Sawyer *et al.*, 1980) caused a pronounced increase in pigmentation (unpublished data).

DISCUSSION

The choice of enzyme for the separation of epidermis and dermis is important. Since collagenase attacks the basal lamina and the dermal collagen fibrils, fibroblasts are often set free and can

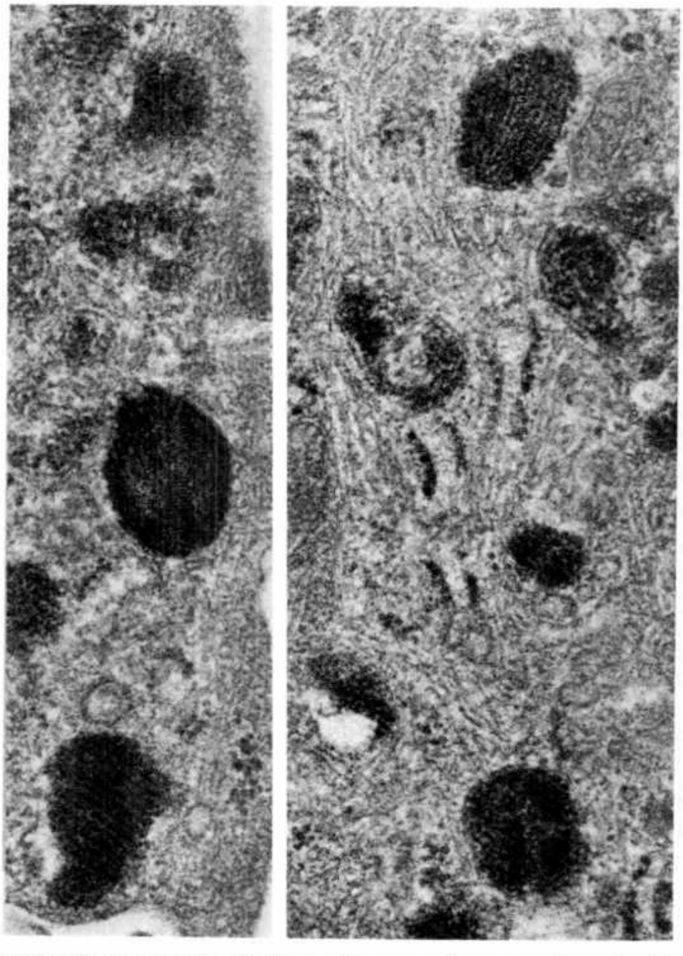

FIGURE 8. Electron micrographs of cells from a human melanocyte culture showing melanosomes (× 62,500).

contaminate the epidermal cell suspension later obtained by treatment with DTE and trypsin (cf. Brooks, Godefroi & Simpson, 1962). Trypsin flotation, on the other hand, affects the skin on the epidermal side of the basal lamina and fibroblast contamination is easier to avoid. Thus, in our hands, trypsin flotation proved superior to collagenase treatment, being faster and more reliable and producing more viable melanocytes with a lower risk of fibroblast contamination.

Goldenhersh *et al.* (1982) and Kobayasi *et al.* (personal communication) used Percoll gradient centrifugation to separate the different epidermal cell types with the emphasis on the isolation of keratinocytes. We attempted to use Percoll gradients to separate and isolate melanocytes from the other cell types.

In preliminary experiments with discontinuous Percoll gradient centrifugation we found that upon cultivation, layer 1 (1·050) contained the most keratinized keratinocytes, layer 2 (1·065) the majority of the melanocytes and layer 3 (the sediment) the most viable keratinocytes. However, it must be concluded that Percoll gradient centrifugation is not an effective means of obtaining an enriched melanocyte culture, since variations in yield proved to be too high. Further elaboration of the technique might decrease this variation, which may be partly due to differences in the origin of skin. Nevertheless, the sediment (layer 3) almost consistently yielded the smallest percentage of melanocytes and was often used for establishment of keratinocyte cultures.

Using a differential adhesion technique, an easy and efficient method of obtaining melanocyte cultures was the seeding of single-cell suspensions of non-separated epidermal cells in FIB41B medium with a low content of Mg^{2+} and Ca^{2+}. This largely inhibited the attachment of keratinocytes. In agreement with this, Patel *et al.* (1981) found that both these ions influenced the attachment of murine epidermal cells, while according to Fritsch *et al.* (1979a) the adhesion of guinea-pig keratinocytes was Mg^{2+}-dependent and Ca^{2+}-independent.

Conditioning of the growth medium by fibroblasts did enhance the growth of melanocytes, but in agreement with the results of Melbye and Karasek (1973) and Yuspa *et al.* (1981) it also stimulated the growth of keratinocytes. Thus fibroblast conditioning was not helpful in the establishment of enriched melanocyte cultures.

Polyamine supplementation of media is known to stimulate the growth of various cell types (Heby, 1981). In this study it was shown that in the presence of fibroblast-conditioned medium supplemented with HSSP the growth of melanocytes exceeded that of keratinocytes, thus contributing to the enrichment of the melanocyte culture.

In conclusion, enriched adult human melanocyte cultures can be obtained by seeding epidermal cells in Mg^{2+}- and Ca^{2+}-free medium for 24–48 hours, and then shifting to regular medium containing 10% fetal calf serum or still better to fibroblast-conditioned medium containing horse serum, spermine, spermidine and putrescine.

A sure and positive identification of all cells in a culture is difficult, due to the low number of premelanosomes often found in the melanocytes and some degree of dedifferentiation. The identification was therefore based on the presence of premelanosomes in some cells and a comparison of the overall ultrastructural appearance of these and other cells. The identification was confirmed by the reaction of the cells to cholera toxin and in some cases to melanocyte stimulating hormone.

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Autonomic function in cholinergic urticaria and atopic eczema

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SUMMARY

Autonomic function in patients with cholinergic urticaria and atopic eczema was compared with that in patients with chronic idiopathic urticaria and non-atopic allergic contact dermatitis, respectively. Forty-nine patients were studied. The following specific tests of sympathetic and parasympathetic function were used to assess the integrity of autonomic control: pupillary reflex amplitude in response to pulsed light stimulation and response to topical arecoline hydrobromide (parasympathetic); the speed of pupillary dilatation following a large light reflex (sympathetic); pupillary resting diameter (sympathetic and parasympathetic); sweat gland response to forearm intradermal injection of acetylcholine chloride; and the effect of postural change and deep breathing on blood pressure and heart rate (sinus arrhythmia indicating parasympathetic function). No significant difference in autonomic function was found between the four groups of patients.

Cholinergic urticaria is associated with an exaggerated cutaneous response to cholinoceptor stimulation, the well-known exercise effect being mimicked by intradermal injection of acetylcholine or its derivatives (Grant, Bruce Pearson & Comeau, 1935). In addition, some patients develop exercise-induced asthma which may be blocked by atropine-like drugs (Blumberg, 1978; Soter *et al.*, 1980). Similarly, atopic patients may develop bronchospasm with exercise and sweat gland responses have been variably reported as exaggerated or normal (Kaliner, 1976; Kiistala, 1982). Pupillary cholinergic hyper-responsiveness was found in patients with asthma and allergic rhinitis, as well as in individuals with positive skin tests (Smith, Shelhamer & Kaliner, 1980). In view of evidence pointing towards a generalized cholinoreceptor supersensitivity, we studied autonomic function in cholinergic urticaria and atopic eczema comparing these two groups with chronic idiopathic urticaria and non-atopic allergic contact dermatitis respectively, two conditions in which no disturbance in autonomic function has been reported.

This work was reported in an abbreviated form at the ESDR Clincally Oriented Symposium 'Brain and Skin', Amsterdam, February 1983.

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METHODS

Forty-nine patients, aged between 18 and 47 years, were studied. Thirteen patients (mean age 29·9 years) had cholinergic urticaria, diagnosed on clinical grounds. Twelve patients (mean age 30·5 years) had chronic urticaria of unknown cause, as defined by Champion (1969). Twelve patients (mean age 27·0 years) had atopic eczema fulfilling the Rajka criteria (Hanifin & Rajka, 1980). All of these patients had positive skin prick tests, as did three patients in both the cholinergic and chronic urticaria groups. Two patients were excluded from the atopic eczema group as they had taken antihistamines on the morning of investigation. No patient included in the analysis of results had taken antihistamines or drugs affecting autonomic function in the preceding 48 hours. Twelve patients (mean age 29·7 years) had contact dermatitis. Atopy was excluded on the basis of an absent personal or family history of atopic disorders, negative skin prick tests and normal serum IgE. One patient was excluded from this group as she had conjunctivitis on the day of examination (see Table 1). Three patients in the atopic eczema group had asthma. Normal skin was used for the sweat gland tests. None of the patients had a florid rash at the time of testing. The tests were carried out in an air-conditioned room at 20°C.

TABLE 1. Details of patients studied

	No. of patients	Mean age	Atopic	Sex
Cholinergic urticaria	13	29·9	3	7M/6F
Chronic urticaria	12	30·5	3	6M/6F
Atopic eczema	10	27·0	10	5M/5F
Contact dermatitis	11	29·7	0	6M/5F

Pupillary measurements. These were performed using infra-red television pupillometry (Smith & Smith, 1978). The aim was to measure the following: resting darkness pupil diameter, an indicator of sympathetic and parasympathetic integrity; light reflex amplitude and sensitivity to a cholinomimetic miotic (parasympathetic tests); and the speed of redilatation following a large light reflex response which tests sympathetic integrity. The tests were performed as follows.

(1) After 30 minutes of dark adaptation achieved by wearing red goggles, the vertical pupillary diameter in one eye was measured in the dark with the subject focussing on infinity.

(2) Five 0·5 second light flashes of an intensity giving approximately half-maximal pupillary reflex responses were directed at one eye every 8 s. The last four reflexes were averaged to obtain the mean reflex amplitude and the resting diameter at the foot of the reflex. Two 1 s flashes of brighter intensity giving near maximal pupillary reflex response were given at 12 s intervals. from the second response, the time from the peak constriction to three-quarters maximal dilatation ($t_{\frac{3}{4}}$) was measured.

(3) Arecoline, a fast-acting pilocarpine-like drug, was used to assess sensitivity to a cholinomimetic miotic (Smith & Smith, 1978). Drug solutions were freshly prepared in isotonic phosphate buffer each day and warmed to 34°C before instillation with a Pasteur pipette. Two drops (each of 0·03 ml) of arecoline hydrobromide 0·01% were instilled into one conjunctival

sac. Serial measurements of the changing pupillary diameter were obtained until a plateau was reached, usually within 12 minutes after application of the eye drops.

Sweat gland test. The number of sweat glands responding to forearm intradermal injection of acetylcholine chloride 1 μg (5·5 nmol) in a fixed area of 42 mm^2, outlined by a template, was counted using the plastic impression technique of Thomson & Sutarman (1953), as described by Clubley *et al.* (1978). Four sites were injected on each patient's forearm. After 1 minute a plastic graphite solution was painted over the injection site. After 2 minutes the plastic impression was stripped off with sellotape and mounted on a glass slide which was coded both for patient and position on the arm. Sweat gland counts were performed on three of the four injected sites; the fourth was observed to detect any abnormal response. Projection of the slide on a screen at the time of counting facilitated the counts, which were done on a blind basis by one person (G.M.M.), after the investigations were completed on all patients.

Cardiovascular studies. Heart rate and blood pressure variability were assessed using a computer-based technique (Smith & Smith, 1981). R–R intervals were measured with the patient lying quietly at rest in the supine position, then passively tilted to 70° upright and then supine again. Simultaneous blood pressure measurements were obtained by sphygmomanometry. The R–R intervals were recorded via lead 2 of the electrocardiogram, converted to digital form and fed on line to a Commodore Pet Series 2001 32K computer for analysis. Successive R–R intervals were then recorded for 255 beats with the patient lying at rest. The standard deviation of these intervals is a measure of sinus arrhythmia. Finally, sinus arrhthymia was exaggerated by deep breathing at six cycles per minute. The longest and shortest R–R intervals during five successive inspirations and expirations were averaged and the mean difference was recorded.

Statistical analysis. Analysis of variance was used to assess the influences of sex, age, disease and other independent variables on the results of the tests of autonomic function.

RESULTS

Age and sex significantly affected the pupillary results. Males had more dilated pupils than females ($P < 0{\cdot}05$). Resting pupil diameter, reflex constriction with light, recovery after light stimulation and arecoline-induced miosis did not differ between the groups (Table 2). Three

TABLE 2. Pupillometry and sweat gland stimulation

	Resting pupil diameter (mm)	Light reflex amplitude (mm)	Redilatation $t_{\frac{3}{4}}$ (s)	Arecoline effect (mm)	No. of active sweat glands
Cholinergic urticaria	6·93 ± 0·21	1·07 ± 0·11	2·93 ± 0·15	0·92 ± 0·18	84 ± 7·7*
Chronic urticaria	7·13 ± 0·19	1·10 ± 0·12	2·57 ± 0·27	0·74 ± 0·12	97 ± 11·0
Atopic eczema	6·96 ± 0·25	1·20 ± 0·13	2·67 ± 0·29	0·90 ± 0·30	99 ± 9·3
Contact dermatitis	6·94 ± 0·[illegible]	1·04 ± 0·12	2·33 ± 0·25	0·70 ± 0·12	87 ± 11·8

All values represent the mean (± s.e.m.) for each group of patients studied.

* Mean + s.e.m. number of sweat glands activated by 5·5 nmol acetylcholine in 3 × 42 mm^2 forearm skin.

patients with asthma and atopic eczema did not appear to differ significantly from the remainder.

Sweat gland stimulation (Table 2). Statistical analysis revealed no difference between diseases. Males in all groups sweated more than females ($P < 0{\cdot}001$). The number of sweat glands activated increased ($P < 0{\cdot}001$) in sites near the wrist compared with sites near the antecubital fossa, a difference of 50%.

Cardiovascular studies (Table 3). No significant differences were found between diseases. Males had a higher resting blood pressure than females ($P < 0{\cdot}001$). Increasing age had a significant effect on changes in heart rate, provoked by sinus arrhythmia, and postural change, in that the older patients had less variation in heart rate ($P < 0{\cdot}05$). As the four groups were well matched for age and sex, these findings did not influence the results for individual diseases.

TABLE 3. Cardiovascular studies

	Cholinergic urticaria	Chronic urticaria	Atopic eczema	Contact dermatitis
Mean BP (mm Hg)	89·5 ± 1·9	87·1 ± 3·4	87·5 ± 2·7	90·2 ± 2·9
ΔBP (mm Hg)	8·6 ± 1·3	9·1 ± 1·2	8·3 ± 1·6	8·7 ± 1·7
ΔHR (beats/min)	18·0 ± 2·4	15·7 ± 2·2	14·9 ± 2·3	17·0 ± 1·6
Mean RR (millisec)	943 ± 30	881 ± 50	905 ± 44	906 ± 44
SDRR (millisec)	74 ± 8·6	62 ± 4·0	58 ± 7·7	61 ± 8·1
SDRR O/E ratio	1·18 ± 0·12	1·15 ± 0·10	0·96 ± 0·12	1·04 ± 0·14
DBRR (millisec)	388 ± 44	363 ± 35	363 ± 36	327 ± 33
DBRR O/E ratio	1·12 ± 0·12	1·12 ± 0·13	1·07 ± 0·09	1·08 ± 0·17

All values represent the mean (± s.e.m.) for each group of patients studied.
Mean BP = Diastolic blood pressure + 1/3 pulse pressure.
HR = Heart rate.
RR = R–R interval on electrocardiograph.
SDRR = Standard deviation of R–R interval.
DBRR = R–R interval on deep breathing.
O/E = Observed/expected.
ΔHR = The change in heart rate on tilting.
ΔBP = The change in blood pressure on tilting.

DISCUSSION

Elevated body temperature, exercise, emotion and gustatory sweating lead to precipitation of cholinergic urticaria (Herxheimer, 1955). Sweating is not an essential feature, as cholinergic urticaria has been described in acquired anhidrosis (Kay & Maibach, 1969). Histamine is released from mast cells and in some patients an elevation of venous blood histamine concentration may occur as the rash develops (Soter *et al.*, 1980). The role of acetylcholine remains ill-defined. As well as acetylcholine, vasoactive intestinal polypeptide and neurotensin have been identified in the nerve endings supplying the vascular plexus of the sweat glands and it is possible that they too play a part in the release of histamine (Hartshuh, Weihe & Reinecke, 1983). The sequence of events leading to the appearance of the lesions of cholinergic urticaria remains speculative. Possibilities include a lowered threshold at receptor level to normal amounts of acetycholine or, as has recently been demonstrated in one paper, an increased number of specific muscarinic receptors, thereby amplifying the pharmacological histamine-released effect of acetylcholine (Shelley, Shelley & Ho, 1983). Alternatively, impaired cholinesterase activity may lead to localized high concentrations of acetylcholine. Our results argue against the concept of a generalized cholinoceptor supersensitivity underlying the disease. Study of the resting pupil diameter, light reflex amplitude and sensitivity to arecoline (a cholinomimetic miotic), amplitude of sinus arrhythmia, resting heart rate, tachycardia on tilting and sweating and response to acetylcholine, failed to reveal any difference between the two groups.

The results obtained in atopic eczema are interesting, as opinions on whether sweating is increased or decreased in atopic dermatitis are contradictory (Kaliner, 1976; Kiistala, 1982). Kiistala studied quantitative sweat gland responses using an evaporimeter, and found no difference between normal subjects and atopic dermatitis. Kaliner did not study atopic dermatitis *per se*; therefore it is difficult to compare his findings of exaggerated pupillary responses and sweating in atopic patients with our study, in which we detected no difference in sweating in atopic eczema compared with the other groups.

Sweating was observed to be markedly increased distally on the forearm by acetylcholine. This is not surprising as sweat glands are greatly increased on the palms to augment grip and there is an increase in the number of active sweat glands distributed distally on the forearm compared with more proximal areas both at rest and after provocation by a hot bath (G.M.M., personal observation). Thus it is important to compare similar sites on the arm when assessing sweating. Sweating is increased by testosterone (Burton *et al.*, 1972), and it is to be expected therefore that males sweat more than females.

The cardiovascular studies indicated that male patients had a higher resting blood pressure than females, and older patients had less variation in heart rate on tilting and deep breathing.

The influence of age and sex on tests of autonomic function is important. There is a wide variation in the normal population between young and old, and between males and females. As our groups were well matched with regard to these variables, these findings did not influence our group comparison.

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Cutaneous and pulmonary reactivity in cholinergic urticaria

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SUMMARY

Cutaneous and pulmonary reactions to mediators of inflammation were studied in twenty-one patients with cholinergic urticaria, in twenty patients with other types of urticaria, and in seventeen healthy controls. Compared with controls and patients with moderate disease, patients severely affected by cholinergic urticaria or other types of urticaria developed larger weals at sites of injection with histamine (9×10^{-7} M), but not with compound 48/80 (0·1%), acetylcholine (7×10^{-5} M), kallikrein (4×10^{-10} M) or ATP (10^{-6} M). On bronchial challenge with acetylcholine (7×10^{-5} M), pulmonary resistance increased markedly only in severely affected cholinergic urticaria patients. There was no correlation between cutaneous and pulmonary reactivity in individual subjects. Patients with cholinergic urticaria therefore demonstrate features of heightened skin reactivity (shared by patients with other urticarias) and an increased bronchial reactivity (as found in latent asthmatics).

Cholinergic urticaria (CU) is a non-IgE-mediated type of urticaria that is provoked by physical excercise, passive overheating and mental stress (Warin & Champion, 1974; Illig & Heinicke, 1967). The mechanisms underlying the production of the pin-point size, transient weals are poorly understood. Herxheimer (1956) has provided evidence for an involvement of cholinergic nerve fibres in the wealing response, but a direct pathogenetic relationship to acetylcholine (ACh) or a unique wealing response to intracutaneous ACh injections could not be demonstrated (Commens & Greaves, 1978). Many aspects of the disease, such as the responsiveness of the cutaneous mast cells and the neurovascular tissue to mediators of inflammation and neurotransmitters, have not been explored.

In the present investigation, we have studied the following aspects of the pathogenesis of CU. (1) Cutaneous reactivity was studied after intracutaneous injections of mediators of inflammation and neurotransmitters in CU patients, in patients with other types of urticaria and in healthy controls. (2) The relationship of the skin reactions to disease severity was assessed. (3)

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Since exercise-induced asthma has been described in patients with CU (Soter *et al.*, 1980), pulmonary function tests were performed on the majority of subjects before and after challenge with ACh.

METHODS

Subjects

Twenty-one patients with CU, twenty patients with other types of urticaria and seventeen healthy controls participated in the study. The groups did not differ significantly with regard to age, sex, incidence of allergic disease or smoking habits. Each patient group was further segregated into a severely and mildly affected disease category, based on the frequency and severity of their symptoms (Table 1). Among the severely affected patients with other types of urticaria, three had acute, six chronic, one light-induced and one dermographic urticaria, while among the moderately affected patients with the other urticarias, five had chronic, two had cold-induced and two had dermographic urticarias. None of the patients had taken any medications known to influence their disease within 72 h prior to testing.

Skin tests

In preliminary studies, several dilutions of the test substances were tested, and the concentrations yielding medium size weals and flares were further studied, namely, 50 μl of histamine (9×10^{-7} M), compound 48/80 (0·1%), kallikrein (4×10^{-10} M), ATP (10^{-6} M), (all from Sigma, Munich, F.R.G.) and ACh (7×10^{-5} M) (Dispersa, Baeschlen, Germina, F.R.G.), all diluted in pyrogen-free saline. These were injected intracutaneously on the upper back of all subjects. Ten minutes later, the diameters of weal and erythema (mm) were read in two dimensions at 90° angles, and results were expressed as the product of these measurements.

Pulmonary function test

Twenty of the twenty-one patients with cholinergic urticaria, twelve of the twenty patients with other urticarias and fourteen of the seventeen controls agreed to have pulmonary function studies performed. The following values were obtained by body plethysmography: vital capacity, total capacity, residual volume, intrathoracic gas volume, resistance and specific resistance. Immediately after inhalation of 1 ml of a nebulized (7×10^{-5} M) acetylcholine solution, the same measurements were repeated.

The statistical significance of all data was evaluated with the Student's *t*-test, and results are expressed as mean ± 1 s.d.

RESULTS

On analysis of the skin test data, no statistically significant differences were found between the cholinergic and the other urticaria group and the controls for any of the test agents. After the patients were regrouped according to disease severity (Table 1), the sizes of weals in response to histamine were markedly increased in severely affected subjects of both patient groups, compared with the moderately affected patients and the controls (Table 2). This did not hold for the weals in response to kallikrein, ACh, ATP, and compound 48/80. The sizes or the erythema reactions varied widely within the groups, and statistically significant differences were not noted with any of the substances.

Although there was no cutaneous hyper-reactivity that might be specific for CU patients,

TABLE 1. Composition of the test groups*

	Mean duration of disease (years)	Number
Cholinergic urticaria		
Severe (at least once daily)	5·0	11
Moderate (less than once daily)	4·3	10
Other urticarias		
Severe (at least once daily)	3·3	11
Moderate (less than once daily)	2·1	9
Controls	—	17

* There were no significant differences with regard to age, sex, history of allergy or smoking habits between the patients and controls, nor did the patients differ with respect to the duration of their disease.

TABLE 2. Weal and erythema reactions after intracutaneous injections of test substances*

Test substance	CU (21)		Other urticarias (20)		
	Severe (11)	Moderate (10)	Severe (11)	Moderate (9)	Controls (17)
Weal					
Histamine	352 ± 128	227 ± 59	371 ± 187	224 ± 87	280 ± 45
48/80	399 ± 218	256 ± 89	381 ± 339	228 ± 73	310 ± 128
Kallikrein	193 + 49	178 ± 34	209 ± 138	187 ± 79	202 ± 38
ACh	96 ± 45	118 ± 62	101 ± 69	108 ± 31	102 ± 31
ATP	87 ± 26	80 ± 16	102 ± 61	83 ± 29	101 ± 22
Erythema					
Histamine	2471 ± 1158	2327 ± 958	2912 ± 1145	2663 ± 1267	2764 ± 721
48/40	2529 ± 1044	2160 ± 1040	2027 ± 1060	1871 ± 915	2659 ± 956
Kallikrein	1537 ± 725	1499 ± 1025	1621 ± 881	1301 ± 595	1771 ± 617
ACh	956 ± 564	788 ± 422	879 ± 474	974 ± 355	1063 ± 416
ATP	1064 ± 522	817 ± 508	1138 ± 919	1085 ± 540	1373 ± 495

* Results are expressed as the product of two measurements of the diameter of the lesions (mm), ± 1 s.d. The differences of the histamine weal between severe and moderate CU ($P<0\cdot005$), and between severe and moderate other urticarias ($P<0\cdot05$) were statistically significant.

there was an increased pulmonary reactivity to ACh with CU patients, compared with patients with other urticarias and controls (Table 3). The increases in pulmonary resistance and specific resistance were more marked in the severely affected patient group. There was no correlation between the skin reactions to ACh or histamine and the pulmonary reactions to ACh in the individual patient. Baseline values of the pulmonary function tests did not differ between any of the groups.

TABLE 3. Pulmonary function after ACh provocation*

	Mean rise in specific resistance (SR_{aw})
Cholinergic urticaria (20)	7·32 ± 1·70
Severe (11)	10·66 ± 0·15
Moderate (9)	2·04 ± 0·06
Other urticarias (12)	4·25 ± 5·49
Severe (7)	4·81 ± 5·41
Moderate (5)	3·23 ± 3·71
Controls (14)	3·54 ± 4·05

* The mean rise of the specific pulmonary resistance (cm H_2O per sec) is shown before and after ACh challenge. The differences are highly significant between CU patients and controls ($P < 0{\cdot}01$), and between severely affected CU patients and patients with other urticarias ($P < 0{\cdot}001$).

DISCUSSION

The data reported in this study show that in patients with CU, there was no specific cutaneous hyper-reactivity to any of a broad battery of mediators. The patients reacted similarly to the healthy control subjects and to patients with other urticarias. CU patients who were severely affected developed increased weals on injection with histamine, but this was also observed in patients severely affected by other urticarias. This heightened cutaneous reactivity was not found with ACh, kallikrein and ATP reactions which were of about equal size in all test subjects.

Kallikrein and ATP have not previously been tested systematically in patients with CU, and the tests seemed attractive since both mediators have been implicated in the pathogenesis of some specific types of urticaria (Kaplan, 1979). ATP is secreted, together with ACh, from cholinergic nerve endings (Morel & Meunier, 1981) which are clearly involved in the production of the cholinergic weals (Herxheimer, 1956). Both ACh and ATP can cause mast cell histamine release *in vitro* (Fantozzi *et al.*, 1978; Diamant & Krüger, 1967) and *in vivo* (Schmutzler, Glück, Huber, 1983; Hägermark, Diamant & Dahlquist, 1974) in normal and allergic subjects. Our present data are in agreement with these findings, but since the skin reactions of CU or other urticaria patients differ neither in size nor quality from those of controls, an increased sensitivity of patient mast cells to these mediators can be ruled out. These findings do not exclude the possibility that cholinergic nerve endings of CU patients secrete larger quantities of ACh, ATP or an additional, as yet unknown mediator. Another possibility is the increase of cutaneous receptors in CU skin which has recently been observed in the affected but not the normal skin of one patient (Shelley, Shelley & Ho, 1983). We should like to see this interesting observation confirmed in other patients and have an explanation offered why this increase is not present in normal patient skin.

The heightened pulmonary reactivity we observed, might be due to an increased vagotonus which is also found in patients with latent asthma (Parker, Bilbo & Reed, 1965). There is however no indication that CU patients tend to develop asthma more readily than do other patients, and our patients with severe CU did not complain of pulmonary symptoms during exercise challenge. The heightened reactivity of CU patients to ACh may therefore have no clinical implications but it may be related to the, as yet unexplained, exercise-induced asthma with associated CU (Soter *et al.*, 1980) and the exercise-induced anaphylaxis (Sheffer *et al.*, 1983) which can also be associated with CU (Kaplan *et al.*, 1981).

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Correlations between human skin bacteria and skin lipids

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SUMMARY

Correlation matrices were constructed for various human skin variables (propionibacteria, micrococcaceae, sebum excretion rate, percentage of free fatty acids and free fatty acid production rate). Two independent populations were studied ($n=87$ and $n=103$), and a subpopulation ($n=30$) was investigated on three occasions at intervals of 1 month. In this way consistently high correlations could be differentiated from 'fortuitous' correlations. Propionibacteria versus micrococcaceae showed the highest correlation coefficient and propionibacteria versus percentage free fatty acids was also highly correlated. A scatter diagram indicated a relationship between the lower threshold of microbial density and sebum excretion rate. An explanation based on relative duct and gland size is presented.

The factors controlling the density of micro-organisms on the skin and the effects of microbes on skin chemistry and pathology are important to many dermatological conditions and their treatments (Noble, 1981) but the published data is fragmentary (Cove, Cunliffe & Holland, 1980; Cove, Holland & Cunliffe, 1980; Webster, Ruggieri & McGinley, 1981; Pochi & Strauss, 1964; Marples *et al.*, 1970; Marples, Downing & Kligman, 1971; Cotterill *et al.*, 1972; McGinley *et al.*, 1980). One way to determine relationships between variables of interest is to carry out correlation analysis. A significant correlation coefficient between variables, although not proving a causal relationship, is nevertheless an indication that such a relationship may exist. Correlations between all variables of interest should preferably be carried out on a single large population to facilitate comparison of coefficients. Even so, it is possible to obtain 'nonsense' correlations (Sokal & Rohlf, 1969), or real correlations existing only within the observed population or on the particular sampling occasion, i.e. sensitive to altered conditions. To detect robust relationships, correlation matrices must be constructed for different populations and on different sampling occasions. Any reproducible correlation within such matrices would be indicative of a robust relationship.

In this study we have examined correlation matrices for the following skin variables: sebum

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excretion rate (SER); percentage of free fatty acids in skin surface lipid (FFA); production rate of FFA; density of propionibacteria and density of micrococcaceae. Two independent populations were surveyed and a smaller subpopulation was studied on three separate occasions. In addition, a relationship between sebum excretion rate and the density of skin micro-organisms was detected only by examining the scatter diagrams for these two variables, illustrating the usefulness of such graphical representations.

METHODS

Subjects. 1981 survey: fifty males and thirty-seven females between the ages of 17 and 25 years were involved. The subgroup used for temporal studies consisted of eighteen males and twelve females. Skin variables were measured at monthly intervals on three occasions. 1982 survey: seventy-seven males and twenty-six females aged between 18 and 21 years were involved. All subjects had mild or moderate acne.

Bacteriological sampling. Bacteria were sampled from an area on the face of each subject by the 'scrub' technique (Williamson & Kligman, 1965). Colony forming units were determined by plating out serial decimal dilutions of the wash fluid onto appropriate recovery media, described previously (Cove, Cunliffe & Holland, 1980). Bacterial numbers were expressed as $\log_{10}$ colony-forming units per cm^2 of skin.

Sebum excretion rate. The SER was determined using absorbant papers (Strauss & Pochi, 1961) as modified by Cunliffe & Shuster (1969) and was expressed as ng sebum cm^{-2} skin·min^{-1}.

Free fatty acid analysis. Sebum extracted from the absorbant papers was reconstituted in 5 ml *n*-heptane and titrated against a weak alkali (N/200 tetra-*n*-butyl ammonium hydroxide in redistilled methanol) using an autoburette (Radiometer, Copenhagen). Results were expressed in two ways, as a percentage of the total sebum and as a production rate of FFA expressed as ng FFA cm^{-2} skin·min^{-1}.

RESULTS

Mean values for all variables measured, together with 95% confidence limits and range are presented for the 1981 and 1982 studies in Tables 1a and 1b, respectively. There were no significant differences in mean values obtained between the two studies for any of the variables, and only small differences from previously published data (Cove, Holland & Cunliffe, 1980). The range of values was also similar in the two populations.

The correlation matrix for the 1981 survey (Table 2) revealed the highest correlation between densities of propionibacteria and micrococcaceae, followed by the FFA indices against propionibacteria and micrococcaceae. Similar correlations were seen between SER versus propionibacteria, SER versus micrococcaceae and SER versus percentage FFA.

The 1982 population matrix (Table 3) showed similar results. The propionibacteria versus micrococcaceae correlation coefficient was again the largest and propionibacteria were again correlated with the FFA indices. However, micrococcaceae were not correlated with FFA indices. This suggests that the correlation observed between these two variables in the 1981 data may have been indirect, resulting from the high FFA versus propionibacteria and propionibacteria versus micrococcaceae correlations. The smaller coefficients between SER and both propionibacteria and micrococcaceae were again seen in this population.

TABLE 1. The mean, 95% confidence limits and range for each skin variable, in each population survey

Variable	Mean ± 95% confidence limits	Range	Units
(a) 1981 Data			
Propionibacteria	4·94 ± 0·32	7·28–1·20	Log_{10}CFU* cm^{-2}
Micrococcaceae	4·53 ± 0·20	6·20–2·00	Log_{10}CFU* cm^{-2}
Sebum excretion rate (SER)	950 ± 80	2280–320	ng cm^{-2} min^{-1}
% Free fatty acids (FFA)	9·84 ± 1·37	25·84–0·96	%
Production rate FFA	100 ± 20	530–10	ng cm^{-2} min^{-1}
(b) 1982 Data			
Propionibacteria	5·26 ± 0·26	7·43–2·26	Log_{10}CFU* cm^{-2}
Micrococcaceae	4·73 ± 0·18	6·46–1·86	Log_{10}CFU* cm^{-2}
Sebum excretion rate (SER)	890 ± 60	1440–340	ng cm^{-2} min^{-1}
% Free fatty acids (FFA)	10·54 ± 1·44	32·73–0·78	%
Production rate FFA	100 ± 20	470–10	ng cm^{-2} min^{-1}

* CFU = colony forming unit.

TABLE 2. Correlation matrix for skin parameters measured in survey I (1981) [$n = 87$]

	Propionibacteria	Micrococcaceae	SER	% FFA	FFA production rate
Propionibacteria	1·00				
Micrococcaceae	0·62**	1·00			
SER	0·35**	0·26*	1·00		
% FFA	0·57**	0·52**	0·26*	1·00	
FFA production rate	0·56**	0·46**	—	—	1·00

* $P < 0·05$.
** $P < 0·01$.
All other correlations were not significant.

TABLE 3. Correlation matrix for skin parameters measured in survey II (1982) [$n = 103$]

	Propionibacteria	Micrococcaceae	SER	% FFA	FFA production rate
Propionibacteria	1·00				
Micrococcaceae	0·59**	1·00			
SER	0·30**	0·31**	1·00		
% FFA	0·32**	0·08	0·22*	1·00	
FFA production rate	0·39**	0·19	—	—	1·00

* $P < 0·05$.
** $P < 0·01$.
All other correlations were not significant.

TABLE 4. Correlation coefficients determined at monthly intervals for a subgroup of population I ($n = 30$)

		Visit 1	Visit 2	Visit 3
Propionibacteria *versus*	Micrococcaceae	0·68**	0·67**	0·57**
	SER	0·05	0·10	0·20
	% FFA	0·54**	0·50**	0·57**
	FFA production rate	0·53**	0·40*	0·52**
Micrococcaceae *versus*	SER	0·17	0·18	0·45**
	% FFA	0·47**	0·26	0·39*
	FFA production rate	0·46**	0·25	0·46**
SER *versus*	% FFA	0·10	0·46**	0·16

* $P < 0{\cdot}05$.
** $P < 0{\cdot}01$.
All other correlations were not significant.

The results obtained for the subgroup sampled on three separate occasions are presented in Table 4. The largest and most reproducible correlations were between propionibacteria and both micrococcaceae and FFA indices. All other comparisons produced low or variable coefficients.

Although the linear correlation between SER and skin micro-organisms was low as measured by the correlation coefficient, there was nevertheless a relationship between SER and the lower

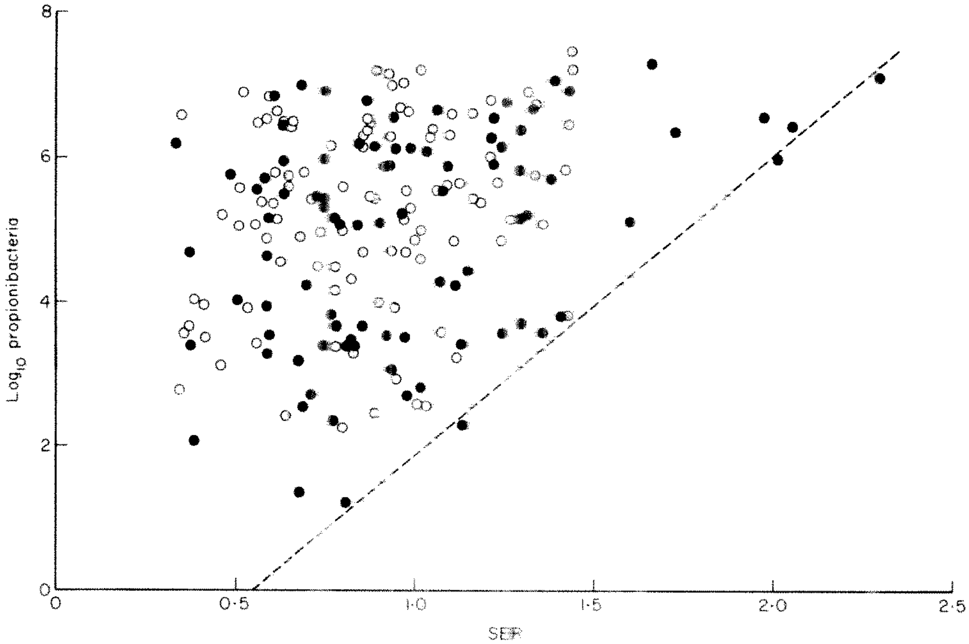

FIGURE 1. Scatter diagram for $\log_{10}$ propionibacteria against SER. The fitted (by eye) line depicts a linear relationship between the lower limit of bacterial density and SER. ● 1981 data. ○ 1982 data.

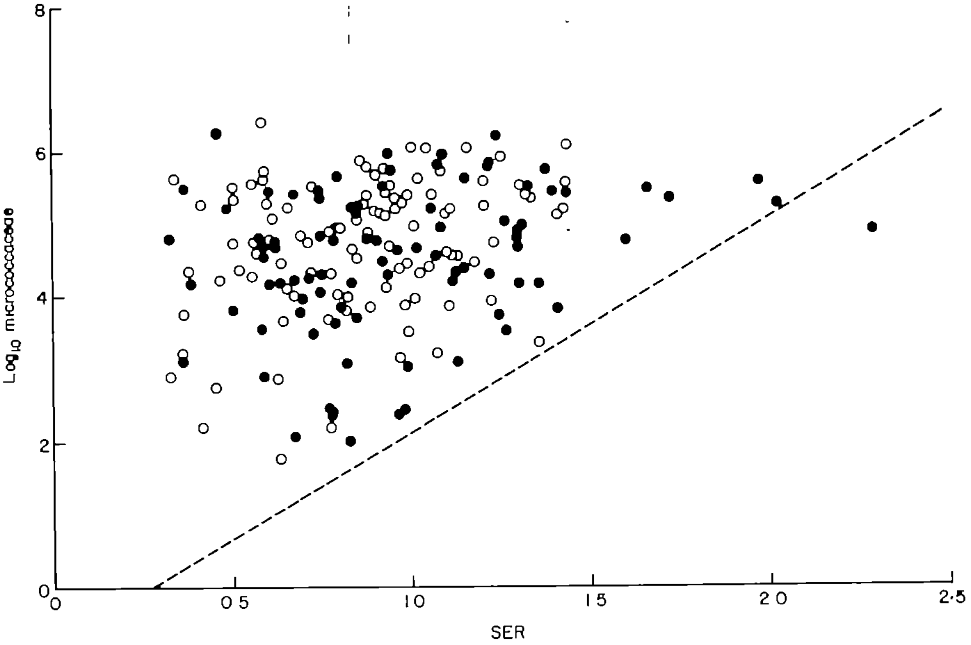

FIGURE 2 Scatter diagram for log$_{10}$ micrococcaceae against SER. The fitted (by eye) line depicts a linear relationship between the lower limit of bacterial density and SER. ● 1981 data. ○ 1982 data.

level of microbial density readily observed on the scatter diagrams (Figs 1 and 2). The dotted lines demonstrate that the lower limit of microbial density rises with increasing SER, i.e. at high SERs the microbial density is always high whereas at low SERs the density may be either high or low.

DISCUSSION

Some of the correlation coefficients in this study are small; however they should not be discounted, particularly when shown to be reproducible. This is because any errors involved in measuring skin variables *in vivo* may decrease the coefficients of existing real correlations. The gravimetric measurement of SER has been shown to reflect not only sebum production, but also sebum storage capacity (Cunliffe & Shuster, 1969; Downing, Stranieri & Strauss, 1982) which differs between individuals. Likewise, the proportion of skin bacteria removed by the scrub technique has been shown to vary from person to person (Aly, Maibach & Bloom, 1978). This would introduce errors in the assessment of correlations involving either value. It is important to note that the largest coefficient between propionibacteria and micrococcaceae was for two variables sampled simultaneously by the same technique, thus perhaps avoiding sampling heterogeneity. This would be particularly true if both microbial groups had similar follicular distributions (Kearney *et al.*, 1984).

A second major correlation between propionibacteria and FFA indices has been observed previously (Marples *et al.*, 1970, 1971; Cove, Holland & Cunliffe, 1980) and has been explained by the action of propionibacterial lipases on triglycerides. This is interesting in view of the relationship between propionibacteria on the skin of mammals and the presence of triglycerides

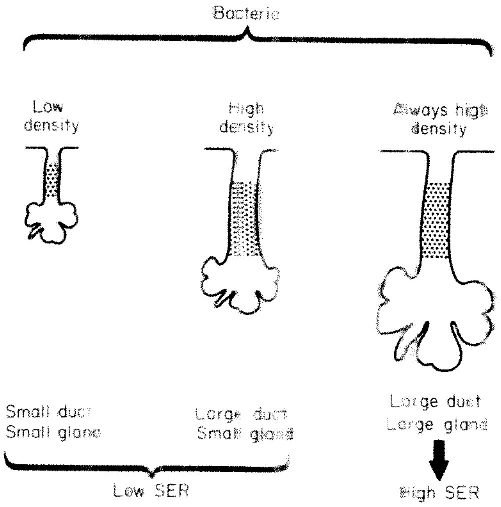

FIGURE 3. Schematic representation of follicle size and relative sebaceous gland size, and how they could affect sebum excretion rate and bacterial density.

in their sebum (Webster *et al.*, 1981) and suggests that some product of triglyceride cleavage may be essential to propionibacteria.

The final correlation of interest is between SER and both propionibacteria and micrococcaceae, seen in both populations of subjects, although not present in a previous study (Cove, Holland & Cunliffe, 1980). This correlation was not present in the smaller subgroup of subjects; however the scatter diagram indicates that the linear relationship only becomes apparent at the higher levels of SER. A larger sample size would have more points in this region. The reason for the increase in the lower limit of microbial density with increasing SER is unclear. A nutritional effect seems unlikely in view of the high densities of bacteria obtained even when the SER is very low. It is more likely to be an indirect effect. One possibility is that small sebaceous follicles always possess small sebaceous glands and hence low SER, whereas large follicles may have either large or small glands and consequently exhibit a range of SER from high to low (Fig. 3). It follows that high SER would be indicative of large sebaceous follicles. Assuming that bacteria are more numerous in large follicles, then it can be expected that high SER will be associated with high bacterial numbers whereas low SER may result from either large or small follicles and consequently contain either large or small populations of bacteria. Differences in the size of follicles from different subjects and heterogeneity in gland size have been observed (J. Leeming, personal communication) and the hypothesis is therefore testable. This could also explain the high correlation between propionibacteria and micrococcaceae if both have similar follicular distributions. There is evidence that this is the case (Kearney *et al.*, 1984).

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Intercellular junctions and the permeability barrier in human sebaceous glands

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SUMMARY

Human sebaceous glands were studied by electron microscopy using lanthanum tracer and freeze-fracture methods. Gap junctions but no tight junctions were found. It is suggested that the essential permeability barrier may be formed by the secreted sebaceous neutral lipid.

In human sebaceous glands, both tight junctions (Cashion, Skobe & Nalbandian, 1969) and gap junctions (Kitson, van Lennep & Young, 1980) have been found. The distinction between these two types of junction may be made by using intercellular tracers in thin-section preparations and by the freeze-fracture technique. Both methods were used in this study to investigate the nature of the permeability barrier in human sebaceous glands.

METHODS

Normal post-auricular skin was obtained from adult white Australian males undergoing elective middle ear surgery. In the course of these procedures, an elliptical incision was made in the post-auricular crease, and the excised ellipse was obtained for this study. Patients were routinely fasted for 6 hours prior to surgery, and all received a general anaesthetic.

Samples were immersed in fixative (glutaraldehyde 2·5% in 0·1 mol/l sodium cacodylate buffer, pH 7·4) in the operating theatre and left for 24 hours. Further processing for thin sections, using lanthanum as a tracer, has been described (Kitson *et al.*, 1978). For freeze-fracture preparations, samples were removed from fixative to 20% glycerol for 30 min, and then transferred to 40% glycerol. Sebaceous glands were separated from whole, fixed, glycerinated skin with 26 gauge needles. Isolated glands were placed in a drop of 40% glycerol between two clamped, holed, gold discs (Balzers 187 281) and the entire preparation was then frozen in nitrogen slush. Fracturing was accomplished in a Balzers 300 BAF at −115°C by means of a double replica device (Balzers 12020). The films were applied with electron gun evaporators (Balzers EK 552), and replicas were cleaned with chromic acid alternating with sodium hypochlorite. The electron microscope was a Philips 400.

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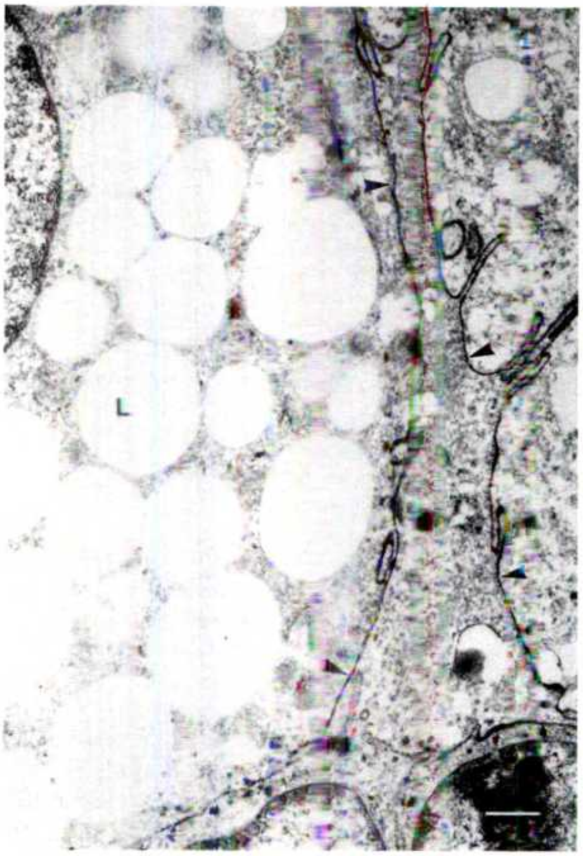

FIGURE 1. Sebaceous cells from a 66-year-old male. Lanthanum (arrowheads) has penetrated the intercellular spaces next to a cell containing many well-developed lipid droplets. (× 6960.) Bar = 1 μm.

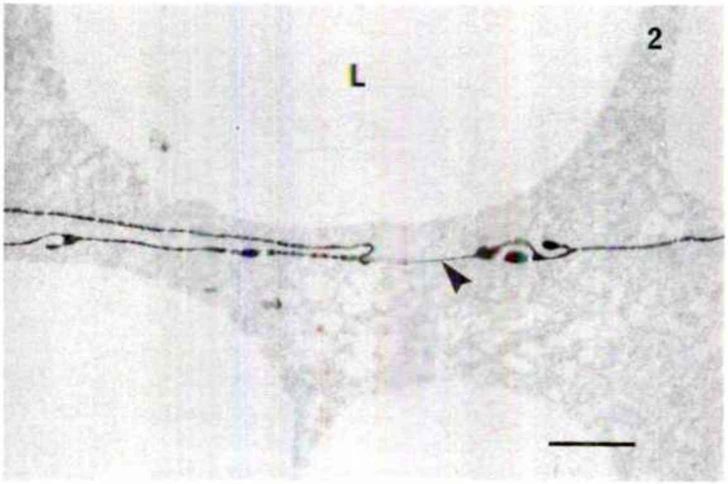

FIGURE 2. Sebaceous cells from a 66-year-old male. Lanthanum has penetrated a gap junction (arrowhead). (× 23,100.) Bar = 0·5 μm.

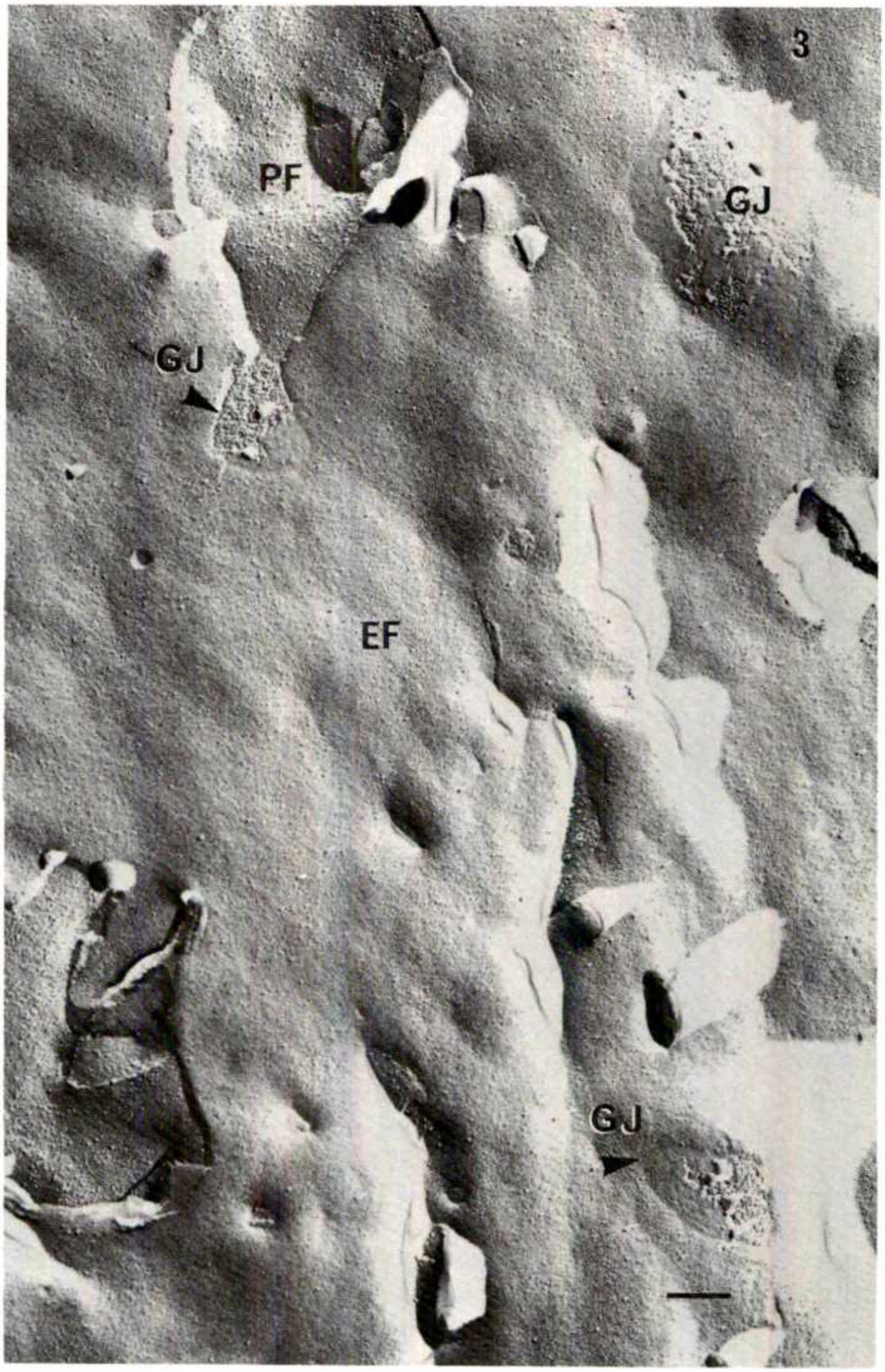

FIGURE 3. Sebaceous cells from a 36-year-old male. Plasma membranes from adjacent cells show three gap junctions (GJ) but no other junctions. The field includes the EF face of the nearer plasma membrane, and the PF face of the further membrane. All these junctions show fracture steps between PF and EF faces. (× 44,800.) Bar = 0·2 μm.

RESULTS

In this study, lanthanum was found around cells containing well developed lipid droplets (Figs 1 and 2), but not within or around the lipid mass following sebaceous cell lysis (Fig. 5). However, lanthanum was found to penetrate the intercellular spaces of the duct cells surrounding the lipid mass (Fig. 5). The progress of the tracer was never found to be impeded by a tight junction.

In freeze-fracture replicas, gap junctions were found to occur frequently (Figs 3 and 4) but typical tight junctions were not observed.

DISCUSSION

Using thin-section and freeze-fracture electron microscopy, evidence has been found for the existence of gap junctions, but not tight junctions, in human sebaceous glands. Gap junctions are thought to play an important role in intercellular communication (Evans, 1980; Revel *et al.*, 1980). Loewenstein and his colleagues have suggested that such communication may regulate cellular proliferation (Loewenstein, 1980): since sebaceous glands function by continuous cellular proliferation, it is possible that gap junctions may play an important role in the regulation of this form of holocrine secretion. It is of interest that, in developing brown adipose tissue of the rat, the size and frequency of gap junctions were found to parallel the rate of lipid metabolism (Schneider-Picard, Carpentier & Orci, 1980). However, there is as yet no firm evidence in any tissue as to the nature of effects of non-electrical signals that pass through gap junctions.

Tight junctions are thought to form an important part of the barrier to water movement across epithelia (Diamond, 1978). However, in mammalian epidermis it appears that tight junctions are rare, and the barrier to intercellular transepidermal water movement may be formed by extracellular lipid (Elias & Friend, 1975; Elias, McNutt & Friend, 1977). The absence of tight junctions from all membranes examined in the present study raises the question of the nature of the permeability barrier in human sebaceous glands, and it must now be considered that, as in epidermis, tight junctions do not form an effective barrier to intercellular water movement.

An obvious alternative is that secreted lipids form a barrier to water exchange between the gland and skin surface. The secretion of neutral lipid by the holocrine process may be considered in physical terms to be the inversion of an oil-in-water emulsion to form a water-in-oil emulsion. While this is a simplification, there must nevertheless be a transition from the cellular part of a sebaceous gland, where lipid droplets are dispersed within an aqueous phase, to the hair or sebaceous follicle, where lipid forms the continuous phase (containing fragments of cellular material). Thus there would be no continuous aqueous phase that would allow water movement between the skin surface and the intercellular spaces of the gland. In the absence of tight junctions, the movement of any molecule from the skin surface to the interstitial spaces of sebaceous glands would presumably depend on solubility in sebaceous gland lipid. Thus it is conceivable that the barrier presented by human sebaceous glands to the environment may differ from that provided by epidermis.

ACKNOWLEDGMENTS

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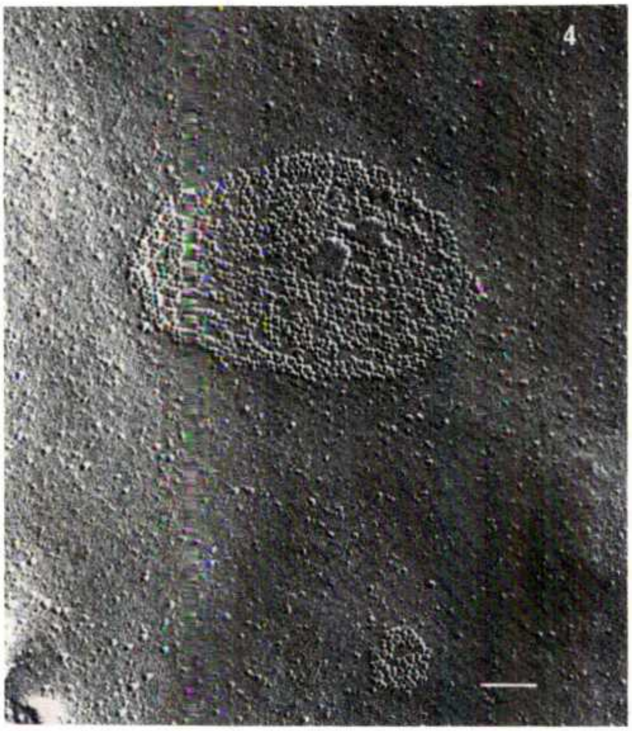

FIGURE 4. Sebaceous cell plasma membrane from a 36-year-old male. The field shows two gap junctions in the EF face. Two small areas of the overlying PF face remain in the larger junction. (× 72,410.) Bar = 0·1 μm.

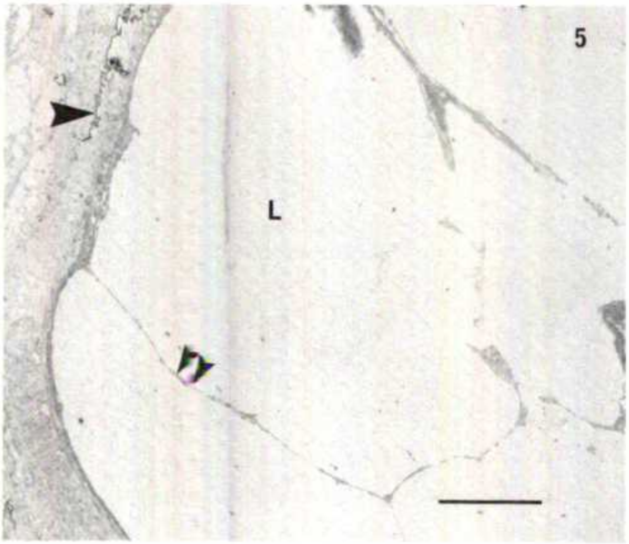

FIGURE 5. Sebaceous cells from a 66-year-old male. The mass of lipid (L) contains no apparent lanthanum either at the periphery or within remnants of cellular material (double arrowheads). Lanthanum can be seen between cells of the surrounding duct (large arrowhead). (× 2700.) Bar = 5 μm.

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Brief Communications

Increased epidermal hyaluronic acid synthesis caused by four retinoids

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SUMMARY

Epidermal hyaluronic acid synthesis in pig skin is increased by all-*trans*-retinoic acid, all-*trans*-retinyl acetate, 13-*cis*-retinoic acid and etretinate.

Although the mechanism by which retinoids modulate epithelial differentiation is not yet clear, an effect on the synthesis of complex carbohydrates is likely to be involved (Lotan, 1980). We showed previously that all-*trans*-retinoic acid (10^{-5} M) increased the incorporation of D-[^{3}H]glucosamine into hyaluronic acid, the major extracellular glycosaminoglycan synthesized in the epidermis of cultured pig ear skin slices (King, 1981; King & Tabiowo, 1981). Kinetic studies suggested that this was due to increased synthesis rather than an effect on degradation. In this report we now show that three derivatives of all-*trans*-retinoic acid, namely all-*trans*-retinyl acetate, 13-*cis*-retinoic acid and etretinate (an aromatic retinoid) have similar, though less pronounced effects on epidermal hyaluronic acid synthesis.

A total of fifty-five keratotomed pig ear skin slices (1 cm^2) were cultured for 18 hours in Eagle's minimum essential medium containing 10% fetal calf serum, gentamicin (5 μg/ml), fungizone (5 μg/ml), and 10 μCi/ml D-[6-^{3}H]glucosamine hydrochloride (35 Ci/mmol) as described previously (King & Tabiowo, 1981). Ethanol or freshly prepared retinoid (10^{-5} M) in ethanol was added to the medium (5 μl/ml). The epidermis was separated from the dermis by dispase treatment. After trypsinization and addition of hyaluronic acid (100 μg/ml) as carrier, glycosaminoglycans were isolated from the extracellular fraction and were separated by electrophoresis on cellulose acetate (King, 1981). The hyaluronic acid band, detected by Alcian blue staining, was cut out and dissolved in 80% acetic acid before measurement of radioactivity.

As shown in Table 1, the greatest stimulation of epidermal hyaluronic acid synthesis was observed with all-*trans*-retinoic acid; 13-*cis*-retinoic acid and all-*trans*-retinyl acetate were equally effective. The aromatic derivative (etretinate) was the least effective of the four retinoids.

The relative effects of these four retinoids on epidermal hyaluronic acid synthesis correlates reasonably well with their activity in several other biological test systems. In the well-characterized hamster trachea organ culture assay all-*trans*-retinoic acid and 13-*cis*-retinoic acid were

TABLE 1. Effect of retinoids on hyaluronic acid synthesis in epidermis of cultured pig skin slices

Addition to medium	Incorporation of D-[^{3}H]glucosamine into epidermal hyaluronic acid: d.p.m. × 10^{-4}/cm^2 of epidermis (mean ± s.e.m. 11 samples)	Retinoid/control
None	9·0 ± 1·5	—
All-*trans*-retinoic acid	25·8 ± 4·6	2·87
All-*trans*-retinyl acetate	19·2 ± 4·2	2·13
13-*cis*-retinoic acid	18·7 ± 3·8	2·07
Etretinate	13·0 ± 2·3	1·44

equally effective at reversing the squamous metaplasia caused by vitamin A deficiency (Newton, Henderson & Sporn, 1980). As in the present work etretinate was less effective. This positive correlation would suggest that the effects of retinoids on epidermal hyaluronic acid synthesis described in this report are related to their influence over epidermal differentiation rather than reflecting any non-specific cytotoxic effect. Increased synthesis of extracellular hyaluronic acid may therefore be of importance for the therapeutic activity of retinoids (particularly 13-*cis*-retinoic acid and etretinate) in a variety of disorders affecting epidermal differentiation (Orfanos, 1980). In this respect it is interesting to note that high levels of hyaluronic acid have been shown to inhibit the differentiation of some embryonic tissues (Kosher & Savage, 1981).

ACKNOWLEDGMENT

I am grateful to Dr A.J.Miller of Roche Products Ltd. Welwyn Garden City, for providing samples of 13-*cis*-retinoic acid and etretinate.

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Circulating androgens in male acne

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SUMMARY

Total serum testosterone, free testosterone, sex hormone binding globulin, albumin and dehydroepiandrosterone sulphate were measured in thirty-five men with severe acne. No striking differences were demonstrated between patients and controls in any of the measurements.

As studies of circulating androgens in acne have been conflicting, we decided to investigate again the circulating androgens and their blood carriers, sex hormone binding globulin (SHBG) and albumin.

Almost all studies have been carried out with women, despite the fact that acne is more severe in men. We have therefore compared thirty-five male acne patients with twenty-three controls with respect to the serum concentrations of total and free testosterone, albumin, SHBG and dehydroepiandrosterone sulphate (DHEA-S).

METHODS

The study group consisted of thirty-five males, twenty-four of whom were suffering from severe papulopustular acne and eleven from conglobate acne. The following determinations were performed.

Testosterone was measured by radioimmunoassay after ether extraction of the plasma. The efficiency of extraction was corrected by addition of tracer [^{3}H]testosterone.

SHBG was determined by ammonium sulphate precipitation, as described by van Kammen *et al.* (1975).

Free testosterone was calculated from the measured testosterone, SHBG and albumin according to Vermeulen, Stoica & Verdonck (1971), taking into account the dissociation constants of testosterone with SHBG and albumin respectively.

DHEA-S was measured directly in diluted plasma by radioimmunoassay using commercially available reagents.

The controls were twenty-three healthy male blood donors of an age-range similar to that of the patients, between 15 and 30 years. DHEA-S levels were not measured in this control group.

Therefore the DHEA-S levels of the patients were compared with the levels of another control group consisting of twelve males without acne between the ages of 15 and 30 years.

RESULTS

Total testosterone levels of the patients and the control group were identical. The mean testosterone level of the patients, 21·4 ± 6·3 nmol/l, agreed with the mean level of the controls, 20·8 ± 7·4 nmol/l. There was also a striking resemblance between the SHBG levels of patients and controls. The mean SHBG of the patients was 23·8 ± 13·2 nmol/l against 23·2 ± 10·1 nmol/l in the control group. In all subjects normal albumin levels were found, ranging from 42 to 50 g/l.

Free testosterone levels of the patients did not differ from the controls. In affected patients free testosterone ranged from 0·17 to 1·02 nmol/l and in controls from 0·14 to 0·93 nmol/l, with means of 0·54 ± 0·18 nmol/l and 0·53 ± 0·19 nmol/l, respectively (Fig. 1).

DHEA-S levels ranged from 2·7 to 19·9 μmol/l in the patients against 3·2 to 12·6 μmol/l in the controls. The mean value found in the patients, 8·8 ± 2·93, was similar to the mean controls, 8·4 ± 1·98 μmol/l.

Statistical analysis assessed by means of Student's independent two-tailed *t*-test did not reveal any differences between patients and controls.

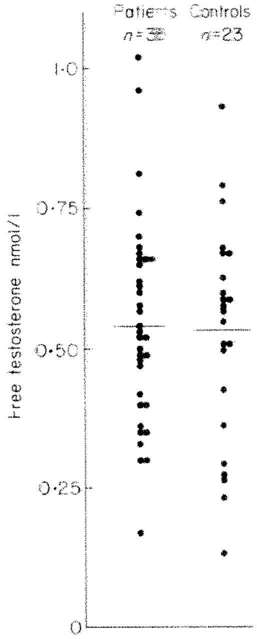

FIGURE 1. Free testosterone levels in male acne patients and controls. Bars = means.

DISCUSSION

Our results indicating that circulating androgens are normal in men with severe acne contradict those of Marynick *et al.* (1983). Although, like us, they found normal total testosterone levels in males, SHBG was present at significantly lower levels than in controls and DHEA-S was increased in 81% of their patients. In female acne patients Darley *et al.* (1982) also found a normal mean testosterone level, but in 26% the levels were above the upper limit of the control range.

Steinberger *et al.* (1981), studying female patients, found an increased mean testosterone level. In male patients we were not able to perceive similar differences. It is difficult to explain the differences between the various studies. One possible explanation could be that with investigations reporting hormonal abnormalities there has been a certain selection towards patients with endocrinological disturbances. It is noteworthy that many studies quoting disturbances in circulating androgens originate from departments of gynecology, reproductive medicine or internal medicine.

Steinberger *et al.* (1981) and Darley *et al.* (1982) have suggested that all females with a chief complaint of acne should be screened for hormonal disturbances. Holshuh (1981) makes firm objections to this. Fanta (1980) concludes that in the overwhelming majority of cases circulating androgens are completely normal.

Endocrinological investigation is rarely performed in men who present with acne at a dermatology out-patient department. This management need not be changed, in our opinion, since we found that there were no abnormalities in circulating androgens and their blood carriers, SHBG and albumin in males with serious acne.

Few studies have focussed on the role of the androgen receptor in acne (Bonne *et al.*, 1977; Schmidt & Spona, 1980). The reasons are manifold: (1) the affinity of the receptor for androgens is only three to five times higher than for SHBG (Kato & Horton, 1968), (2) the receptor is extremely thermolabile (Mowszowicz *et al.*, 1981), (3) there are difficulties in collecting sufficient sebaceous gland tissue. Our study supports the opinion that in the male, acne results from a disturbance localized at the level of the target organ, in or around the sebaceous gland.

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Case Reports

Is hepatoerythropoietic porphyria a homozygous form of porphyria cutanea tarda? Inheritance of uroporphyrinogen decarboxylase deficiency in a Spanish family

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SUMMARY

A patient with hepatoerythropoietic porphyria (HEP) is described. He was shown by a family study to be homozygous for a gene that causes greater than 95% suppression of erythrocyte uroporphyrinogen decarboxylase activity.

Hepatoerythropoietic porphyria (HEP) is a rare form of cutaneous porphyria which presents in early childhood with severe photosensitization associated with excessive excretion of uroporphyrin and other acetate-substituted porphyrins and mild accumulation of protoporphyrin in erythrocytes (Piñol Aguade *et al.*, 1975). Czarnecki (1980) has reviewed the clinical and biochemical features of the seven cases that have been described. Three of these patients have been shown to have a severe deficiency of uroporphyrinogen decarboxylase, with activities in erythrocytes and fibroblasts of less than 10% of normal (Elder *et al.*, 1981). In the father of one of these patients, erythrocyte uroporphyrinogen decarboxylase activity was decreased to the same extent as in the familial form of porphyria cutanea tarda (PCT) (De Verneuil *et al.*, 1978). It was therefore suggested that patients with HEP may be homozygous for the gene that causes this type of PCT (Elder *et al.*, 1981), but it was not possible to study both the parents of any of these cases.

We now report a new case of HEP together with a complete family study showing the pattern of inheritance of the uroporphyrinogen decarboxylase defect.

CASE REPORT

A 2-year-old boy was first seen in 1982 at the 'Hospital Provincial' of Madrid with a history of

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skin lesions in sun-exposed areas. His parents had noticed that during the previous summer he had had similar cutaneous lesions that healed spontaneously, but they did not remember whether there had been any change in the colour of his urine at that time.

Physical examination revealed bullae, scars and erosions on the dorsa of hands, erosions and scars on the face, and pronounced hypertrichosis of the forehead. The teeth were normal and did not fluoresce under Wood's light. The liver and spleen were not palpable. His parents were non-consanguineous and apparently healthy. Both were dark-skinned and the father had some facial hypertrichosis but no cutaneous hyperfragility. The boy's brother and sister, 11 and 8 years old respectively, were both clinically normal.

Results of haematological investigations were: haemoglobin concentration 14·1 g/dl, haematocrit 48%, white cell count 11·8 × 10^9/l (normal differential counts), serum iron 85 μg/dl, serum ferritin 43 ng/ml. Biochemical tests of liver function (serum bilirubin, aspartate and alanine transaminases, bromosulphthalein retention), plasma urea and electrolytes, blood glucose, total serum protein, albumin and IgG, IgA and IgM were all within normal limits. A serum test for hepatitis B surface antigen was negative. A liver biopsy was not performed.

Porphyrin measurements

Table 1 shows the porphyrin concentrations in urine, faeces, erythrocytes and plasma from the patient, measured by a sensitive fluorimetric thin-layer chromatographic technique (Day, De Salamanca & Eales, 1978). The amount of each individual porphyrin present in urine and faeces was expressed as a percentage of the total porphyrin and two 'porphyria cutanea tarda' (PCT) indices were calculated as described by De Salamanca *et al.* (1982). Isomer analysis of urinary porphyrins revealed the following pattern: uroporphyrin (85% type I), heptacarboxyporphyrin (7-COOH) (95% type III), hexacarboxyporphyrin (6-COOH) (89% type III), pentacarboxyporphyrin (5-COOH) (51% type III), coproporphyrin (63% type I). Erythrocyte porphyrin

TABLE 1. Patient's porphyrin analysis

Specimen	Total porphyrins	Individual porphyrins (%)								PCT Index
		URO	7-COOH	6-COOH	OXO	5-COOH	ISO	COPRO	2-COOH	
Urine	11,202	45·8	25·3	8·8	2·0	11·7	3·3	3·1	—	89·1
μg/l	(< 110)	(< 32)	(< 10)	(< 3)	(–)	(< 6)	(–)	(> 55)	(–)	(< 14)
Faeces	88	14·5	14·5	5·9	0·7	8·2	19·9	5·6	30·7	86·0
μg/g dry wt.	(< 90)	(< 4)	(< 1)	(< 2)	(–)	(< 6)	(tr)	(< 42)	(> 50)	(< 4)
Erythrocytes	294	—	—	—	—	—	—	—	100	
μg/dl	(< 40)	(–)	(–)	(–)	(–)	(–)	(–)	(tr)	(> 95)	
Plasma	20	61·7	19·8	14·8	—	3·7	—	—	—	
μg/dl	(tr)	(tr)	(–)	(–)	(–)	(–)	(–)	(–)	(tr)	

Numbers in parentheses give upper limits of normal (mean + 2 s.d.).

OXO = oxo-isocoproporphyrin's R_F band.

ISO = isocoproporphyrin band.

$$\text{Urinary PCT index} = \frac{\text{7-COOH} \times 100}{\text{7-COOH} + \text{COPRO}}$$

$$\text{Faecal PCT index} = \frac{(\text{7-COOH} + \text{ISO}) \times 100}{\text{7-COOH} + \text{ISO} + \text{COPRO}}$$

tr = trace.

TABLE 2. Porphyrin excretion in patient's family

	Total porphyrins µg/l (urine) or g dry wt. (faeces)	Individual porphyrins (%)							PCT Index
		URO	7-COOH	6-COOH	5-COOH	ISO	COPRO	2-COOH	
Father									
Urine	69	29·3	9·9	1·7	4·7	—	54·5	—	15·4
Faeces	10	—	1·2	—	0·6	—	30·2	68·0	3·8
Mother									
Urine	48	15·5	6·5	1·4	7·9	—	68·7	—	8·6
Faeces	32	—	0·7	—	0·5	—	21·1	77·7	3·2
Brother									
Urine	44	17·4	14·9	2·4	2·9	—	62·4	—	19·3
Faeces	54	2·3	0·4	0·3	0·2	tr	7·0	89·8	5·4
Sister									
Urine	45	26·5	3·1	1·2	5·6	—	63·6	—	4·6
Faeces	4	2·8	0·8	3·1	0·5	—	53·2	39·6	1·5

was predominantly zinc-protoporphyrin. Urinary 5-aminolaevulinic acid (1·9 mg/l) and porphobilinogen (1·6 mg/l) excretion were within normal limits.

Urinary and faecal porphyrin analysis for the patient's parents and siblings are shown in Table 2. Minor abnormalities of the urinary porphyrin excretion were found in the father and brother. Urinary porphyrin precursors, erythrocyte and plasma porphyrins were within normal limits for all these relatives.

Enzyme measurements

Erythrocyte uroporphyrinogen decarboxylase activities for the patient and his relatives are shown in Fig. 1. Activities were determined using pentacarboxyporphyrinogen III as substrate (Elder & Wyvill, 1983). Enzyme activities for ten normal subjects were 16·1–30·2 pmol coproporphyrinogen/min/mg haemoglobin (mean 21·6 pmol/min/mg) and for six unrelated patients with familial PCT, 10·3–14·5 (mean 12·5) pmol/min/mg.

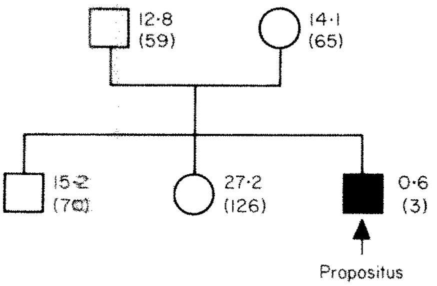

FIGURE 1. Erythrocyte uroporphyrinogen decarboxylase activities. Figures are erythrocyte uroporphyrinogen decarboxylase activities in pmol coproporphyrinogen/min/mg haemoglobin with enzyme activities expressed as a percentage of the mean value for normal controls in parentheses.

DISCUSSION

The differential diagnosis of cutaneous porphyria in early childhood is not always straightforward. In addition to congenital erythropoietic porphyria (Günther's disease) and protoporphyria, at least three other conditions have to be considered: porphyria cutanea tarda, HEP and a syndrome of dyserythropoiesis with partial uroporphyrinogen decarboxylase deficiency that clinically resembles congenital erythropoietic porphyria (Ippen & Fuchs, 1980; Czarnecki, 1980; Day & Strauss, 1982; Kushner *et al.*, 1982). In our patient, the clinical features, absence of anaemia, increased concentrations of uroporphyrin, isocoproporphyrin and porphyrins with five to seven carboxyl groups in urine and faeces (Table 1), and the presence of a mixture of type III porphyrin isomers, together point to a diagnosis of either porphyria cutanea tarda or HEP. The high percentage values of uroporphyrin in the faeces, of pentacarboxyporphyrin in the urine, and the increased erythrocyte zinc-protoporphyrin are more characteristic of HEP than porphyria cutanea tarda (Smith *et al.*, 1982). However the differences in porphyrin excretion patterns between these two conditions are minor and may not always be present (Elder *et al.*, 1981; Day & Strauss, 1982) while the value of an increase in erythrocyte zinc-protoporphyrin concentrations as the sole means of differentiation has been questioned in view of the frequency of iron deficiency and other anaemias which produce a similar increase (Day & Strauss, 1982).

Measurement of erythrocyte uroporphyrinogen decarboxylase activity does, however, appear to distinguish unequivocally between HEP and other porphyrias (Elder *et al.*, 1981). In our patient, and in the three other cases of HEP in which uroporphyrinogen decarboxylase has been measured, erythrocyte enzyme activity was less than 10% of the mean normal value (Fig. 1), thus allowing clear distinction from familial PCT, in which erythrocyte enzyme activity is decreased to about half normal, and from other forms of PCT in which it is normal (Elder *et al.*, 1981).

Complete family studies of HEP have not been reported previously. The parents of our patient both have erythrocyte uroporphyrinogen decarboxylase activities that are approximately 60% of normal (Fig. 1) which suggests that each is heterozygous for a gene that leads to almost complete suppression of enzyme activity. Thus our family study supports the suggestion that patients with HEP are homozygous for this defect (Elder *et al.*, 1981). Current evidence indicates that all the uroporphyrinogen decarboxylase activity of human erythrocytes is present in a single protein (De Verneuil *et al.*, 1983; Elder, Tovey & Sheppard, 1983). It therefore seems likely that the residual enzyme activity present in HEP represents the product of the mutant gene, rather than expression of a gene at a separate locus which codes for an isoenzyme that accounts for about 10% of normal enzyme activity.

Whether the gene defect that gives rise to HEP in homozygotes is the same as that which is present in familial PCT remains an open question. Certainly, our patient's father and brother had minor abnormalities of porphyrin excretion and enzyme levels indistinguishable from those of subclinical PCT (Benedetto, Kushner & Taylor, 1978; De Salamanca *et al.*, 1980). However, clinically manifest porphyria has not been reported in the families of any of the, admittedly few, patients with HEP so far reported.

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Cutaneous lymphomatoid granulomatosis: a rare cause of recurrent skin ulceration

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SUMMARY

We report the case of a 34-year-old male who presented with recurrent skin ulceration due to lymphomatoid granulomatosis. Monoclonal antibody studies showed a high T helper (T4):T suppressor (T8) cell ratio in both the lesions and the peripheral blood. Plasma cells in the infiltrate showed IgA lambda staining but as yet no monoclonal immunoglobulin has been identified in the blood or urine. Lymphocytes bearing markers of immature cells (T10) were observed in the tissue and blood.

CASE REPORT

J.E., a 34-year-old man, presented in January 1980 with a tender, erythematous, ulcerated swelling in the left lumbar region. It had been present for 6 months and there was associated general malaise, night sweats and weight loss over 2 months. During the preceding 2½ years similar lesions had occurred in the left upper anterior abdominal wall, left axilla and on the right shoulder. One had resolved spontaneously and two were excised at another hospital. His past medical history included a detached retina in 1961 and an episode of asthma in 1970.

On examination he was pyrexial, with an area of erythema, induration and central ulceration measuring 23 cm in diameter in the left lumbar region. He had a left third cranial nerve palsy. There was no hepatosplenomegaly or lymphadenopathy. Investigations revealed an elevated ESR (greater than 100 mm/h), WBC $3{\cdot}4 \times 10^9$/l with persistent lymphopenia (2·6 neutrophils, 0·5 lymphocytes, 0·3 monocytes, 0·1 eosinophils), Hb 10·3 g/l, SGOT 100 i.u./l, SGPT 443 i.u./l, γ GT 371 i.u./l, alkaline phosphatase 118 i.u./l. IgG and IgA were both elevated at 30·4 g/l and 4·3 g/l respectively. Chest X-ray showed 'plate' atelectasis and patchy opacification in the lower lobes. Vital capacity was 2·93 litres (69% of predicted), FEV_1 2·06 litres (57% of predicted). Transfer factor was 6·6 mmol min^{-1} kpa^{-1} (79% of predicted). Skin biopsy showed extensive areas of necrosis and only small foci of viable tissue were present. The latter comprised subcutaneous fat with a diffuse lymphohistiocytic cellular infiltrate that included occasional

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multinucleated cells. The necrotic areas showed 'ghost' outlines of medium-sized vessels infiltrated by cells. Differential diagnoses considered included malignant lymphoma, Wegener's granulomatosis, vasculitis and mycobacterial infection but a definitive diagnosis remained uncertain. At that time the possibility of cutaneous lymphomatoid granulomatosis (LYG) was not considered and the histology was reported as a granulomatous lobular panniculitis with septal vasculitis. Stains for infective organisms were negative. A Mantoux test (1 in 1000) and patch test to DNCB were both negative. Syphilis serology and autoantibodies, including rheumatoid factor, ANF, dsDNA, smooth muscle and anti-mitochondrial antibodies were negative.

Other investigations were unremarkable, including liver biopsy, abdominal lymphangiogram, bone marrow trephine biopsy, and culture of sputum, skin and liver for T.B.

Treatment was started empirically with prednisolone 20 mg t.d.s. However, because of gradual deterioration and the remote possibility of mycobacterial infection, he was also given rifampicin, isoniazid and ethambutol. Remarkably, his fever subsided immediately and a few weeks later the ulcerated area had healed. Anti-tuberculous therapy was continued for a total of 9 months, during which time no further lesions appeared. However, he continued to experience intermittent exertional dyspnoea and mild pleuritic chest pain, although chest X-ray appearances remained unchanged. Three months later a new lesion appeared in the left flank. Biopsy again showed extensive necrosis of subcutaneous fat. The fat lobules showed a dense mixed cellular infiltrate composed predominantly of lymphocytes but including histiocytes, small groups of plasmacytoid cells, polymorphs and occasional large atypical mononuclear cells with hyperchromatic nuclei. Multinucleated cells were infrequent and Reed-Sternberg cells were absent. The infiltrate extended into the lower dermis and surrounded the eccrine sweat

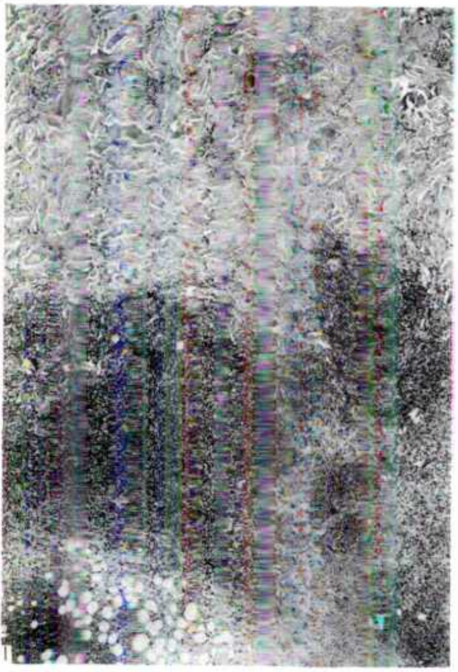

FIGURE 1. Skin biopsy showing a cellular infiltrate in the deep reticular dermis and subcutaneous fat. (H & E, × 80.)

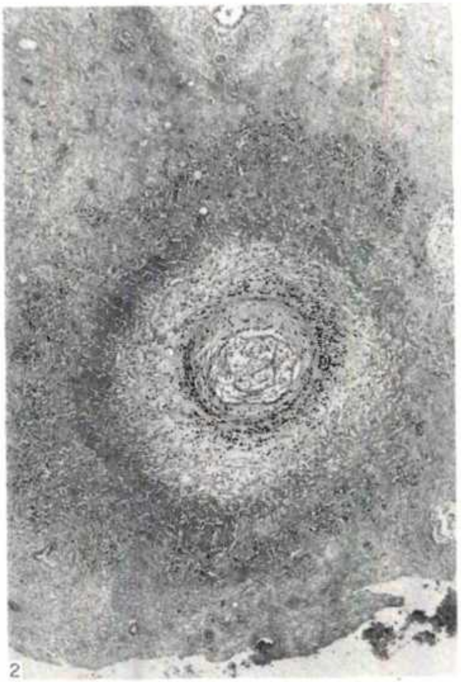

FIGURE 2. Subcutaneous fat showing a large vessel with a cellular infiltrate in its walls and extensive necrosis in the surrounding tissues. (H & E, × 120.)

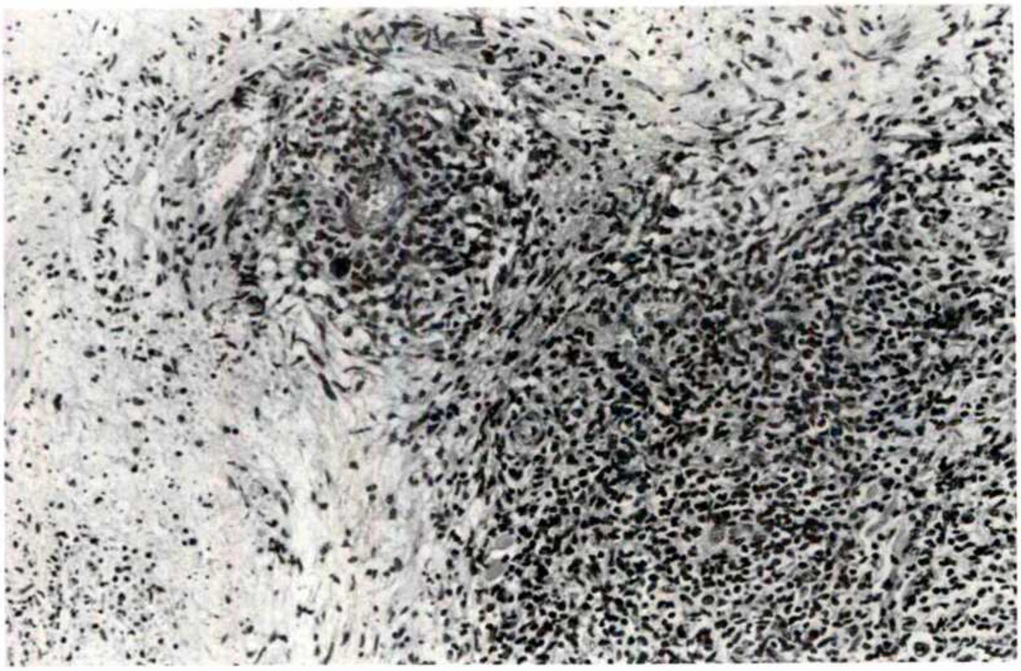

FIGURE 3. Cutaneous cellular infiltrate with occasional large atypical hyperchromatic cells and infiltration of a small venule. (H & E, × 390.)

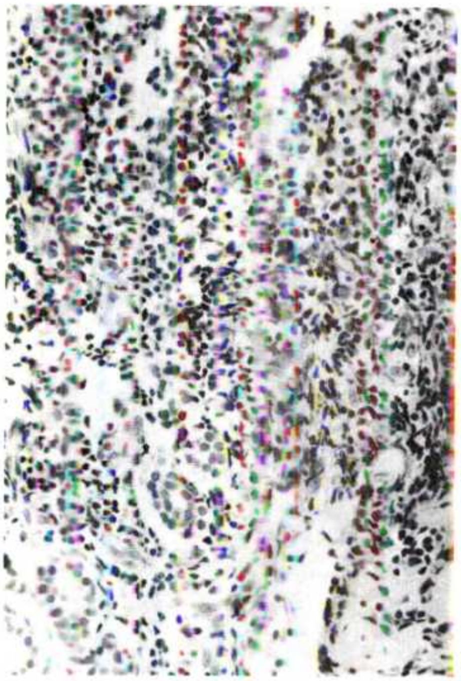

FIGURE 4. Cutaneous lymphohistiocytic infiltrate showing characteristic distribution around sweat glands. (H & E, × 410.)

glands and small nerve fibres. As in the first biopsy the infiltrate involved the walls of small and large veins and arteries (Figs 1–4). There was no evidence of caseation and stains for acid-alcohol fast bacilli were negative.

Again considerable consideration was given to the differential diagnosis. The absence of Reed-Sternberg cells excluded Hodgkin's disease and the appearance was not that of any recognized variant of non-Hodgkin's malignant lymphoma. Infective causes such as mycobacterium infection and syphilis had been reasonably excluded and the presence of atypical cells was unusual for collagen-vascular diseases and the more classical forms of vasculitis (including sarcoidal vasculitis). Atypical cells are not a common feature of Wegener's granulomatosis and the classical variant was excluded by the absence of upper respiratory tract lesions. So-called limited Wegener's granulomatosis was considered unlikely as the granulomatous element of the biopsy was minor. The only known skin disease to display angiocentric and angiodestructive lymphohistiocytic proliferation combined with a polymorphous infiltrate, granulomatous features and atypical cells was lymphomatoid granulomatosis (LYG). Further support for this diagnosis was the common characteristic of involvement of skin appendages—especially the sweat gland (MacDonald & Sarkany, 1976; James, Odom & Katzenstein, 1981). The diagnosis was therefore considered to be that of LYG. Trans-bronchial lung biopsy was attempted but failed to obtain pulmonary tissue. This new skin lesion failed to respond to both anti-tuberculous therapy and oral cyclophosphamide but eventually healed following local radiotherapy (1475 rads on the linear accelerator). Three months later yet another area of induration and ulceration appeared adjacent to the last lesion (Fig. 5).

Local radiotherapy, intermittent high dose cyclophosphamide (1 g i.v. weekly × 3) and

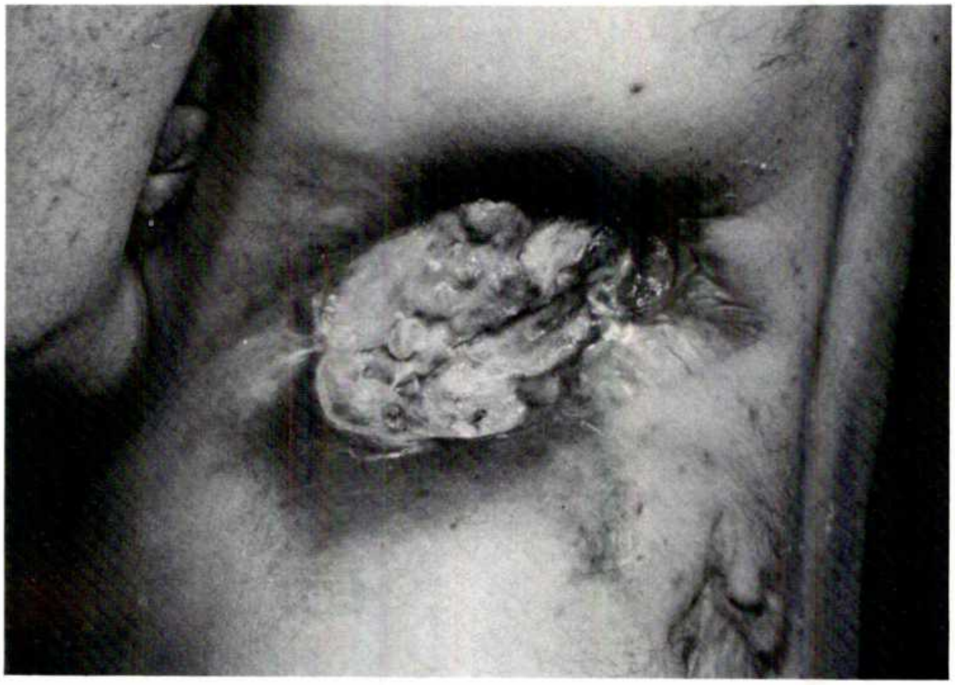

FIGURE 5. Clinical photograph showing a large ulcer in the left loin and a scar from a previous cutaneous lesion below it.

twice-weekly ACTH have had no effect. In January 1983 he developed a new subcutanous mass in the upper anterior abdominal wall which was excised under general anaesthesia. The histological changes were similar to the previous biopsy and again consistent with the diagnosis of LYG. Monoclonal antibody immunoperoxidase studies revealed large numbers of T4 (helper/inducer) lymphocytes with a few T8 (suppressor/cytotoxic) lymphocytes (Fig. 6) and T10 (immature lymphocyte) positive cells were also present. Atypical cells were not identified on the frozen sections and therefore their nature was not characterized. Staining for lysozyme and α_1-antitrypsin showed moderate numbers of histiocytes and the scattered plasma cells present showed only IgA lambda positivity. No monoclonal immunoglobulins or free light chains were detected in the blood or urine, but there was minimal generalized proteinuria of 0·3 g/l. Peripheral blood studies have shown persistent absolute lymphopenia ($< 0{\cdot}5 \times 10^9$/l) with a disproportionately low T-cell count (44%, sheep erythrocyte rosetting) and a high T4 (helper/inducer) to T8 (suppressor/cytotoxic) cell ratio of 4·4:1 which resulted from a low number of peripheral T suppressor cells. In addition a very high proportion (19%) of the peripheral blood lymphocytes showed a marker for immature cells (T10).

DISCUSSION

Lymphomatoid granulomatosis (LYG) was originally described by Liebow, Carrington & Friedman (1972). Histologically it is characterized by an angiocentric and angiodestructive lymphohistiocytic proliferation. The infiltrate is polymorphous with granulomatous features of Wegener's granulomatosis with the cellular atypicality of lymphoma. Progression of the disease to frank lymphoma is well documented occurring in 12–18% of patients (Liebow *et al.*, 1972;

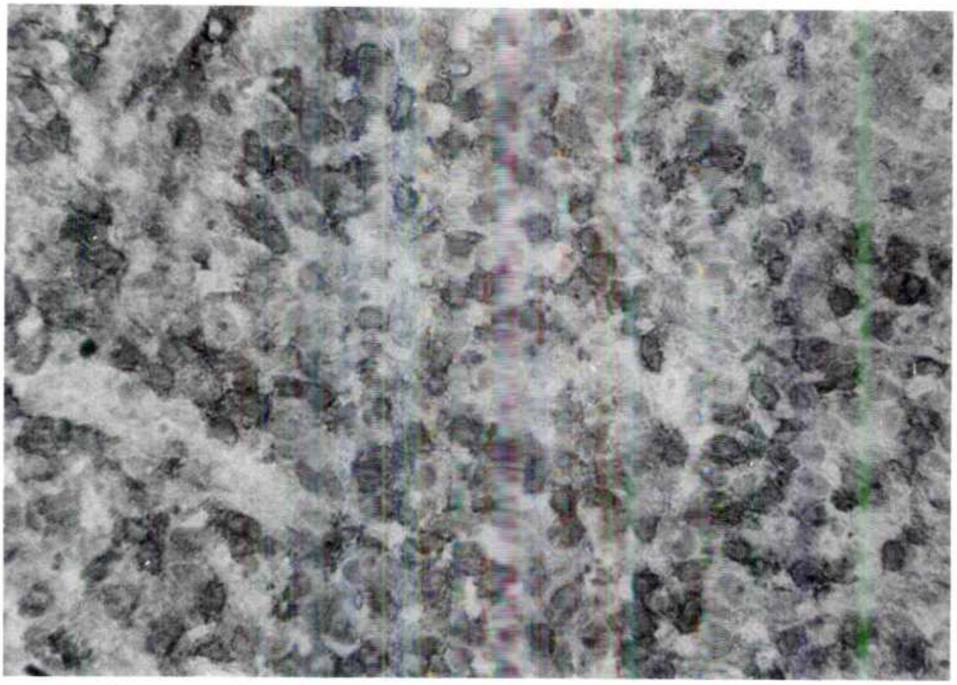

FIGURE 6. Cutaneous cellular infiltrate showing extensive positive staining for T4 (helper/inducer) lymphocytes. (Immunoperoxidase, × 420.)

Katzenstein, Carrington & Liebow, 1979; James et al., 1981) though in one review as many as 47% subsequently developed lymphoma (Fauci et al., 1982). Manifestations of the disease are protean, although pulmonary involvement is the most common. The skin is the next most frequently involved organ, with cutaneous lesions developing in 40–50% of patients, usually in the form of subcutaneous or dermal nodules, occasionally as a maculo-papular rash or macular erythema and rarely as ulceration (James *et al.*, 1981; Holden, Wells & MacDonald, 1982).

Over 180 cases of LYG have now been described, some of which have been associated with unusual dermatological features such as anhidrosis, alopecia, generalized ichthyosis (MacDonald & Sarkany, 1976), vesicular lesions (Kay *et al.*, 1974) and annular cutaneous lesions with central clearing (Bender *et al.*, 1978). Skin lesions may precede (by up to 9 years), occur simultaneously with or follow pulmonary involvement (James *et al.*, 1981). We would stress the importance of searching for skin lesions when the diagnosis of LYG is suspected, as skin biopsy may obviate the need for percutaneous, trans-bronchial or open lung biopsy. However, as experienced in this case, it must be emphasized that the characteristic angio-destructive nature of the disease may result in large areas of ischaemic necrosis, with consequent difficulty in obtaining viable tissue from which to make a diagnosis. Although our case has histological evidence of skin involvement only, the disease is presumably systemic, producing pulmonary and neurological symptoms and signs. Although skin involvement does not appear to affect prognosis, neurological manifestations are said to represent an adverse prognostic factor, median survival being about 14 months regardless of treatment (Katzenstein *et al.*, 1979).

The aetiology of LYG remains obscure. However, the immunological investigations in our case may provide information regarding possible pathogenetic mechanisms. A persistently high T4 (helper/inducer) to T8 (suppressor/cytotoxic) cell ratio in peripheral blood and tissues, due to low numbers of T8 (suppressor/cytotoxic) cells may result in unopposed B-cell stimulation, accounting for the elevation of serum immunoglobulins as present in this case and described in other patients (Sordillo *et al.*, 1982). In addition, the unusually high numbers of T10 lymphocytes in lesional tissue and in peripheral blood lends weight to the idea of a state of unstable immunoregulation and maturation developing in this case.

In view of the presence of plasma cells showing only IgA lambda positivity, it will be of interest in this case to see whether a B-cell malignant lymphoma eventually develops. Such an event would be consistent with the view of immunoblastic lymphoma in LYG being a natural consequence of continued B-cell proliferation (Bender & Jaffe, 1980). However, in contrast to these findings, hypogammaglobulinaemia (Sordillo *et al.*, 1982) and increased T8 (suppressor/-cytotoxic) cell activity (Sordillo *et al.*, 1982; Petras *et al.*, 1982) have been described in other patients with LYG. Consequently, although LYG appears to represent a specific histological entity, different immunological abnormalities may occur.

Until now the treatment of choice has been a combination of prednisolone and cyclophosphamide or radiotherapy and there is evidence that early treatment may prevent relapse or progression to lymphoma (Fauci *et al.*, 1982; and Israel *et al.*, 1977).

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Comment

THE SPECTRUM OF DOWLING–DEGOS DISEASE

Dowling–Degos disease (DDD) has enjoyed a considerable resurgence of interest since Wilson-Jones and Grice drew attention to it in 1978. Eleven more cases have been described (Bardach, 1981; Grosshans *et al.*, 1980; Brown, 1982; Ochiai & Yamaguchi, 1982; Crovato, Nazzari & Rebora, 1983) and its genetic transmission has been elucidated (Brown, 1982; Crovato *et al.*, 1983).

We have noticed an intriguing relationship between DDD and other rare genetic disorders, especially Haber's syndrome (HS) (Crovato & Rebora, 1982; Kikuchi, 1982, 1983) and Kitamura's acropigmentatio reticularis (KAR) (Crovato, Desirello & Rebora, 1983). We believe that DDD is only one facet of a more extensive and complex genodermatosis, including some isolated cases that have previously been recorded under different titles.

The aim of this paper is to propose a spectrum of diseases that present with different clinical features but with a unique histopathological picture of epidermal budding and extensive proliferation of the follicular walls. We will therefore review the reported cases of DDD, HS and KAR, together with those showing similar features, gathering them into a coherent entity.

Clinical features

1. Dowling–Degos disease (DDD) (thirty-four cases). The condition is characterized by deeply pigmented macules, arranged in a reticulate pattern affecting the flexures, inner aspects of thighs, neck and rarely the wrists, scrotum, face and scalp. Small comedo-like hyperkeratotic follicular lesions are often present, along with pitted acneform scars around the mouth, sometimes extending to the entire face. Occasional findings include moderate mental retardation (three cases) and epidermal or trichilemmal cysts (four cases).

2. Haber's Syndrome (HS) (eight cases). These patients have a flushed face with telangiectases. Their follicles are prominent, keratotic or replaced by a pitted scar. Small firm follicular papules are also present.

3. Kitamura's acropigmentatio reticularis (seventeen cases in the Western literature; more than fifty additional cases in Japan). A reticulate slightly depressed pigmentation affects the extensor surfaces of the hands and feet. Milia-sized keratotic papules or simple breaks in the epidermal ridge pattern can be found on palms and fingers.

4. Pigmentatio reticularis faciei and colli with epithelial cystomatosis (HK) (one case in the Western literature and one in Japan) (Hasegawa et al., 1980; Kubota & Hori, 1982). Brown-black pigmented macules in a reticulate pattern are present on preauricular regions, cheek, chin, forehead and neck. Multiple epithelial cysts are found on the chest and back.

5. Familial multiple follicular hamartoma (FFH) (one case) (Delacrétaz & Balsiger, 1979). Miliary whitish cystic nodules affect the face, especially cheek and eyelids, and the chest. Larger cysts occur on the forehead, labia majora, perineum and perianal area.

Histopathology

1. DDD. Narrow epithelial strands extend out from the epidermis and the follicular walls in a filiform pattern, sometimes branching in an antler-like pattern. The Malpighian layer immediately above this is thinner. There are also small intraepidermal cysts and hyperpigmentation of the tips of the down-growths.

2. HS. Epithelial budding results in larger branching formations that originate from both epidermis and follicular walls.

3. KAR. Club-like elongation of the rete ridges and an excess of melanin in the basal layer are associated with slight atrophy of the overlying epidermis.

4. HK. Epidermal, trichilemmal cysts and epidermal inclusion cysts can be found along with atrophy of epidermis and elongation of rete ridges with hyperpigmentation of the basal layer.

5. FFH. Dilated follicles show a thin wall that proliferates in a network of epithelial strands connecting each other and surrounding cystic dilatations.

Genetics

DDD, HS, and KAR have all been shown to be autosomal dominant conditions (Brown, 1982; Crovato *et al.*, 1983; Kikuchi, 1982; Griffiths, 1976), beginning in childhood.

Discussion

One of our patients who had features of both DDD and KAR suggested to us the possibility that these two conditions might be variants of one disease (Crovato *et al.*, 1983). Although the sites of pigmentation are different, DDD being localized mainly to the flexures and KAR to the extensor surfaces of the hands and feet, the histology is very similar. KAR lacks only the antler-like pattern of the epithelial proliferation which, incidentally, is not a constant feature even in DDD.

The five cases described by Becker & Reuter (1939) as familial pigmentary anomaly and the case of Montgomery & Reuter (1932) of xeroderma pigmentosum, later repudiated as such (Becker & Reuter, 1939), were probably genuine cases of this DDD–KAR association. Both of them had reticulate pigmentation of the neck and forearms with a histopathology which was strikingly similar to KAR. The minor clinical and histopathological differences that prevented Wilson-Jones and Grice (1978) from including them in their compilation of DDD cases can be neglected now that the coexistence of DDD with KAR has been shown. For example, atrophy of epidermis and the lack of the antler-like pattern can no longer be regarded as distinguishing points.

The two HK patients are interesting for they associate a reticulate pigmentation suggestive of DDD with a microscopic picture closely resembling KAR. In addition, they had trichilemmal cysts, as had four of the reported DDD cases.

The FFH patient was characterized by trichilemmal cysts alone with a DDD-like histopathology.

HS deserves particular consideration. In fact, the cases published after the original description by Sanderson & Wilson (1965) are questionable. Seiji & Otaki's case (1971) is indeed a case of DDD bearing some similarity with the case of Piñol-Aguadé & Fernandez in that both patients had perioral pitted scars and follicular keratosis. The clinical picture of the patients of Kikuchi, Saita & Inoue (1981) suggests, and Kikuchi himself later admitted (1983), that in fact they had DDD. The same might be true for the other case, reported in Japanese by Yoshinaga *et al.* (1978). Moreover, it is to be noted that Ochiai & Yamaguchi's (1982) DDD patient had a red facies. Also the original HS cases share some features with the patient of Piñol-Aguadé & Fernandez (1973), but lack the reticulate pattern of pigmentation. Moreover, the follicular pits were not perioral but occurred especially on the forehead.

Similar isolated cases may perhaps be found elsewhere in the literature.

Takahashi, Maie & Murai (1982) described a case of acropigmentatio reticularis inversa affecting the palms, soles and buttocks, very similar to KAR but with an epidermal atrophy and flattening of rete ridges that contrast with the features of DDD and KAR.

The patients of Hauss & Oberste-Lehn (1958) and Flegel (1960), by contrast, show many similarities with the patients described, even though they had melanophores in the papillary dermis that do not usually occur in DDD.

Other follicular hamartomas should be kept apart. This applies to the generalized hair follicle hamartoma (Brown, Crounse & Winkelmann, 1969; Ridley & Smith, 1981) to which Delacrétaz & Balsiger (1979) refer, erroneously in our opinion. The patients of Brown *et al.* and the one of Ridley & Smith had follicular hamartoma associated with alopecia and myasthenia gravis. This constitutes a distinct clinical entity with a histopathology that is completely different from DDD, lacking the typical profuse epithelial proliferation of the follicular walls.

The patient described by Mehregan & Hardin (1973) as generalized follicular hamartoma differs from the cases mentioned above in many clinical points and in any case is microscopically quite dissimilar to DDD.

The Birt, Hogg and Dubé (1977) syndrome which consists of fibrofolliculomas, trichodiscomas and acrochordons may have some clinical resemblance with the Delacrétaz & Balsiger patient for it presents with multiple small tumours of the skin. When punctured they express a fluid that suggests a cystic structure. The microscopic picture however is quite different. Long isolated epithelial strands extend out from the hair infundibulum but do not branch as profusely as they do in DDD and are embedded in a specialized fibrous tissue that is definitely lacking in DDD.

Probably, there are other cases that may be taken into consideration. Some of them are included in the exhaustive discussion in the paper of Wilson-Jones & Grice. Others, that may fit our concept of DDD, can be found in the Japanese literature, but are not readily available in English. Nevertheless, the cases herein illustrated seem to be sufficient to delineate the spectrum of DDD. One end of the spectrum is represented by KAR, which clinically shows only reticulate pigmentation, and the other end by FFH, which presents with cysts only. In between, there are DDD–KAR cases, DDD itself and the HK patients, who progressively show an increased prevalence of follicular lesions.

We suggest therefore that the term DDD should be extended to a group of autosomal dominant genodermatoses that is characterized clinically by reticulate pigmented macules, hyperkeratotic follicular lesions or pitted scars and facial erythema, in varying degrees of clinical severity, and histologically by a constant picture of digitate budding and extensive proliferation of the epidermis and follicular walls.

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British Journal of Dermatology (1984) **110**, 631–632.

Obituaries

S. T. Anning

Stephen Towers Anning came from a medical family; his father and two uncles were well-known general practitioners in Leeds and his brother was also a doctor. He started his medical training at Cambridge and returned to Leeds for his clinical work. After qualifying and doing house appointments at the Leeds Infirmary, he went into general practice in Leeds. He had joined the Territorials so that when the war started, he was rapidly called up. He spent most of his service in the Middle East, particularly in Persia and later in Italy, attaining the rank of Lt. Colonel and O.C. Medical Division. After the war he remained for some years on the Army Emergency Reserve, eventually becoming O.C. of No. 27 General Hospital with the rank of full colonel and he was awarded the Territorial Decoration. Whilst he was in the Middle East, he successfully completed an M.D. thesis on desert sores.

After being demobbed he returned for a short time to general practice and decided to specialize. Largely due to the influence of Dr John Ingram, as he then was, he turned to dermatology. At the latter's suggestion he applied for the new University post of Tutor in Dermatology to which he was appointed in 1946. He remained in this post until he was appointed as a consultant physician to the Skin Department in 1950. During these years he had a short spell working with Miescher in Zurich. He brought two attributes to his work in the Skin Department which I feel influenced Ingram in his choice: firstly, his knowledge of subtropical diseases and, probably more important, his experience in general and social medicine gained in general practice outside the hospital. He became the senior dermatologist in 1969 and remained head of the department until he retired in 1973.

As the junior member of the Department's consultant staff, he took over the large 'leg ulcer clinic' and this led eventually to his writing a very useful book on leg ulcers, published in 1954. It was no chance that the first chapter of this book dealt with the history of the subject, for later he went on to write several books and articles on medical history. These included two volumes on the history of the Leeds Infirmary, a history of the Leeds Medical School (which he wrote with Dr Walls for its 150th anniversary in 1982), and other books and articles. His historical contributions were recognized by the University on that occasion by the award of an Honorary Degree of Master of Philosophy. He was also appointed by the Society of Apothecaries to their small and select group of Honorary Lecturers in the History of Medicine and for many years he gave two lectures a year to the students of Leeds on this subject. In 1965 he was appointed the first librarian of the Willan Library at the Royal College of Physicians. He served on the Board of Governors of the Leeds Infirmary and many other committees and was for many years the very conscientious secretary of the Medical Faculty of the hospital. He retired in 1973 and went to Australia for 6 months; on his return he continued in private practice and doing occasional locum work.

Stephen Anning was a very sociable man with a large circle of friends, many of them patients from his days in general practice. He had a happy family life with a son and daughter. Unfortunately his wife, Joan, developed an impairment of vision so that Stephen had to devote much time to her with a care we all admired. To her and to his son and daughter we offer our deepest sympathy.

F.F.H.

K.D.Crow

Kenneth Derek Crow, one of the stars of G.B.Dowling's 'boys', died at his home in Wootton Bassett on 11 January 1984. He was born in London on 12 December 1921, was a scholar of Felsted school and went on to St Mary's Hospital Medical School, qualifying in 1934. He won the Cheadle Prize Medal, and the then Professor of Medicine, the late Sir George Pickering, recognized his outstanding qualities. He did various house jobs, both in his own teaching hospital and at the Royal Postgraduate Medical School. He became a Member of the Royal College of Physicians of London in 1945 and a Fellow in 1969. Unfortunately in 1946, before the discovery of the antituberculous drugs, he contracted tuberculosis, as did so many hospital workers at that time. He was gravely ill and among other things underwent thorocoplasty. After long spells in the Brompton and Midhurst Hospitals he was able to return to work after 4 years. During all this time he was ably supported by his wife Barbara. He turned to dermatology and after various posts at St Mary's and St John's he became Senior Registrar at St Thomas's Hospital in 1953.

Prior to 1957, Dr Alice Carleton, based in Oxford, had also provided a dermatological service to Swindon and district. When she retired, Oxford and Swindon were separated and Ken was appointed to develop the new department at Swindon, embracing Cirencester and Marlborough. He tackled this challenge with enthusiasm. His integrity and administrative abilities were quickly recognized by his colleagues and he was heavily involved in the planning stages of the new Princess Margaret Hospital. A close link was forged with the Oxford Department of Dermatology, which he visited every month, and he became a regular attender at the monthly journal club, making many shrewd comments both on papers discussed and patients presented.

Inevitably, working in an area in which there were many new manufacturing processes as well as old established industries such as the British Railway works, he became involved with both old and new dermatoses associated with industry. His opinion was valued in medico-legal problems but in this respect he will be best remembered for his untiring energy in investigating the causes of peculiar cases he saw in his patients. He was an acknowledged expert on chloracne, and on cable-jointers' dermatoses. He was called in by the Italian Government to advise on the dermatological aspects of the Seveso explosion and he was an invited lecturer on these problems in the U.S.A. and the Far East.

Always a perfectionist, he never spared himself in trying to find the answer to problems with which he was confronted. This was well demonstrated in 1966, when he was the ideal local secretary for the annual meeting of the B.A.D. held in Oxford. The paper he wrote on his experience has been of great assistance to succeeding local secretaries of this annual event.

He was a very keen yachtsman and enthusiastic gardener, and it is sad that although he retired early, he was not able to enjoy his chosen leisure activities. It is not surprising that one of his earlier hobbies was pyrotechnics, since there was never a dull moment when Ken was around. Nobody who has had the privilege of meeting Ken will ever forget him; he was truly a great man and one of the 'characters' of the dermatological world.

RENWICK VICKERS

British Journal of Dermatology (1984) **110,** 633–634.

Correspondence

Clobetasol propionate for PUVA overdosage

Sir, Of course, PUVA overdosage could not occur in your Department, but just in case it does, here is our experience. A 35-year-old male with psoriasis (skin type II) was due to receive his third dose of PUVA therapy, 5 joules. By accident he received 25 joules. The mistake was noticed immediately afterwards as the the patient queried why he had just spent 20 minutes in the light cabinet and only 3 minutes on the previous occasion.

Clobetasol propionate 0·05% (Dermovate®) ointment was applied to the whole body together with stockinette overdressings for 24 hours. No other therapy was given. His skin was observed over the next 72 hours during which it remained normal without blistering or erythema, nor did he itch. He received a further dose of PUVA of 7 joules 2 days later as planned and his psoriasis continued to regress as expected with PUVA therapy. Clobetasol propionate would, therefore, appear to be an effective suppressant for PUVA overdosage if applied immediately.

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C.Hindson
J.Spiro
Barbara Blyth

Leukaemia in psoriatic patients treated with razoxane

Sir, Razoxane has been shown to be an effective treatment for psoriasis (Horton & Wells, 1983). However, two patients treated at Guy's Hospital developed squamous cell carcinomata, and in a recent paper it was recommended that all patients who receive this drug should be observed for the development of malignant disease (Horton, MacDonald & Wells, 1983). Since then two patients treated with razoxane at this hospital have developed acute myeloid leukaemia (type M2, FAB classification; Bennett *et al.*, 1976). Studies of bone marrow chromosomes have revealed cytogenetic abnormalities in both cases.

In 1981 two patients were described who developed acute myelomonocytic leukaemia while taking razoxane for the treatment of malignant disorders (Joshi *et al.*, 1981). Our experience, and other reports, make it necessary to reappraise the place of razoxane in the treatment of psoriasis. In accordance with the manufacturer's recommendations we have suspended its use for the treatment of this condition.

Abnormalities of blood and bone marrow morphology are commonly seen in patients taking razoxane. We are currently carrying out morphological and cytogenetic studies of blood and bone marrow samples from patients who have discontinued treatment, with a view to determining whether potentially leukaemic abnormalities persist.

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Blood transfusion as treatment of erythropoietic protoporphyria

Sir, Drs Dobozy, Csató, Siklósi and Simon (*British Journal of Dermatology* 1983, **109**, 571–576) have treated patients suffering from erythropoietic protoporphyria (EPP) with repeated blood transfusions and they achieved an improved tolerance of sunlight and a rapid reduction of the protoporphyrin levels in the red blood cells. They advocate this therapy in particular for cases with deteriorating liver functions, and thus aim at bringing about a decrease of protoporphyrin accumulation, which is supposed to be the cause of the liver damage.

The above-mentioned authors' advice is to 'consider carefully the risks involved in the treatment'. One of the risks is the development of hepatitis owing to contamination of the transfusion blood with viruses or other microbial agents. Some of the latter may be traced (e.g. hepatitis A or B virus, cytomegalovirus), but others defy detection (non-A, non-B virus). An already existing progressive liver disease and subsequent microbial hepatitis may prove disastrous. In view of this the patient should be subjected to this risk only if definite improvement of the liver condition can be expected. Greater experience is needed to allow better evaluation of the 'pros and cons' of this therapy. If blood transfusion is decided upon for these patients it should be carried out only under careful clinical supervision.

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British Journal of Dermatology (1984) **110,** 635.

Book Reviews

Dermatologie und Venereologie. Leitfaden für Studium und Praxis. G.K. STEIGLEDER (1983), 4th Edition. Stuttgart: Georg Thieme. Pp. 593. Price DM 33.00.

This volume is the fourth revised edition of a popular small textbook primarily aimed at students of dermatology and non-specialist physicians. It is remarkably comprehensive while remaining a 'pocket-book'.

In addition to a formal discussion of most common (and some not so common) skin disorders and sexually transmitted diseases there is a most useful introductory first chapter, and appendices containing suggestions for further reading and a set of multiple choice questions. The book is well illustrated with black-and-white photographs, mostly of excellent quality, and liberal use is made of diagrams. The brief summaries given at the end of each chapter form useful aids to revision and the index is comprehensive and easy to use. Professor Steigleder has also written two companion volumes to this work, a guide to therapy and differential diagnosis, and a pocket atlas of dermatology. Cross reference between the three volumes is made easy by use of a numbered subject index.

Overall, this book is beautifully produced and is excellent value. It will be bought in large numbers by students of dermatology but it should also be recommended to their teachers as it forms an excellent aid to the preparation of lectures and tutorials. An English version of an earlier edition is available and it is hoped that this new edition will also soon appear in translation.

N.SMITH

Stratum Corneum. Edited by R.MARKS and G.PLEWIG (1983) Berlin: Springer-Verlag. Pp. 265. Price DM 64.00.

The stratum corneum may be dead but it has come a long way since Albert Kligman put it on the map with his classical chapter entitled 'The Biology of the Stratum Corneum' in *The Epidermis* (Academic Press, 1964). It is appropriate that one of Kligman's German disciples and a British admirer should have edited this product of a Symposium held in Cardiff in 1981.

Since Kligman's seminal chapter, the stratum corneum has acquired an immunology and a pharmacology which hardly existed 20 years ago. There has been an enormous explosion of knowledge of the anatomical and biochemical nature of this most efficient of nature's barriers which attracts the interest of the detergent and cosmetic industries, the clinical pharmacologist, the psoriasis-addicted and the retinoid chemists with equal allure. All will find worthwhile information in this slim soft-backed volume. All the corneal kings are there. Only the percutaneous administration of drugs for systemic effect is missing—perhaps the most important single consequence of better understanding of the dynamics of percutaneous absorption.

All horny buffs will want this volume within reach.

H.BAKER

L'Herpès, Nouveaux Traitements Nouveaux Espoirs. M. JOSSEY (1983) Paris: Laffont. Pp. 183. Price F 60.00.

I enjoyed reading this little book which is written in excellent and easy flowing French. It is aimed at medical students and general practitioners and I am sure they would be very well informed if they really read it. It has great breadth of content and the treatments go from acyclovir to the liquid obtained from manure: I have no experience of the latter but acyclovir certainly seems to work. One thing this book does is to adopt a hopeful attitude to the treatment, and it may be of great value in dispersing some of the fears of this disease.

SUZANNE ALEXANDER

British Journal of Dermatology (1984) **110**, 636.

News and Notices

Symposium on Hair and Circumscribed Alopecias, Ghent, Belgium, April 1985

An international symposium on Hair and Circumscribed Alopecias will be organized in Ghent, Belgium on 19 April 1985. It will be followed on Saturday, 20 April by a presentation of clinical cases, free communications, and posters. For further information, contact Prof. A. Kint or Prof. J. De Bersaques, Dermatology, Akademisch Ziekenhuis, De Pintelaan 185, B-9000 Ghent, Belgium.

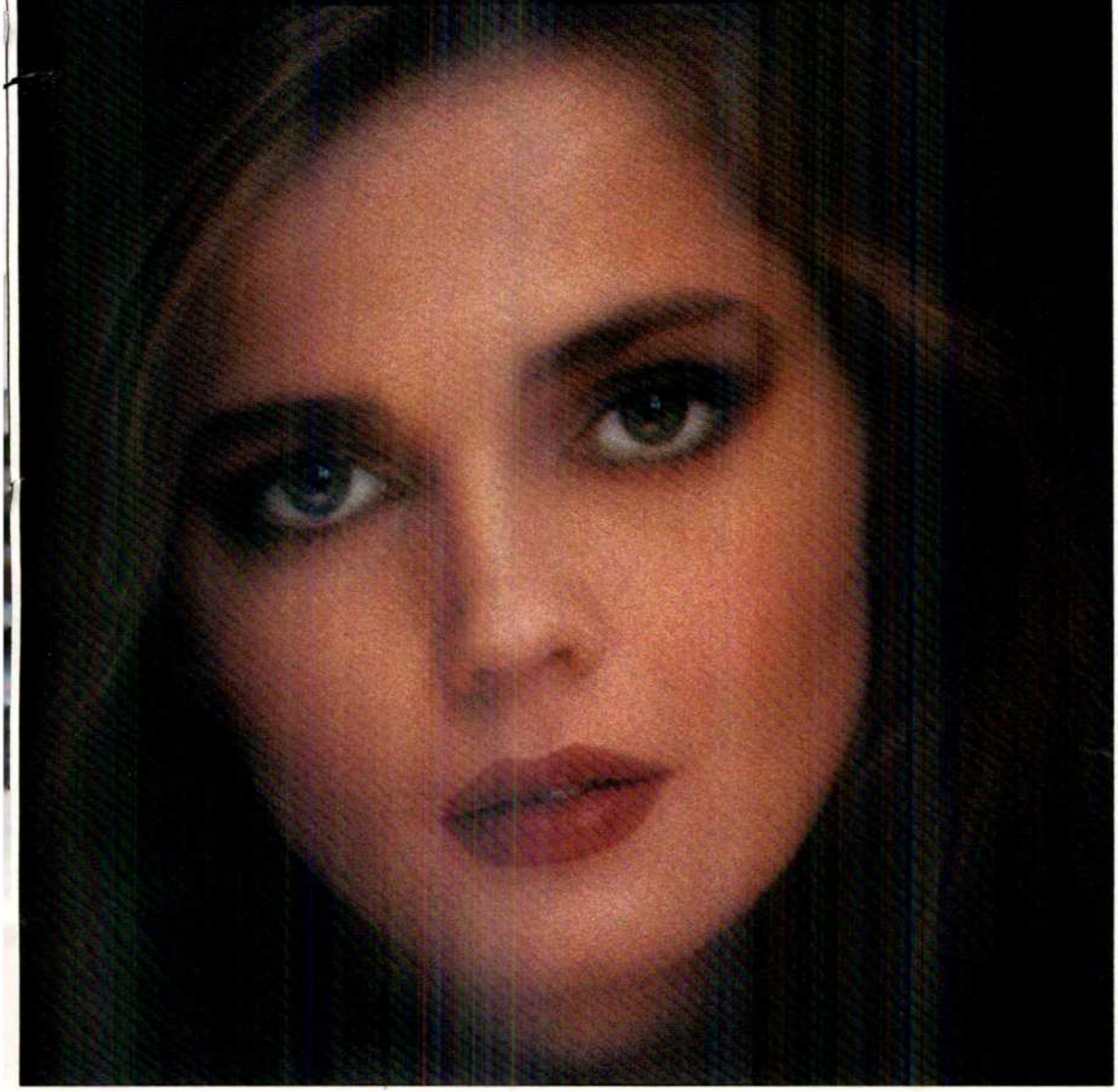
Practical Skin Pathology Course, London, July 1985

This course will be a comprehensive and practical review and update for dermatologists and pathologists. It will include formal lectures, supervised microscope slide workshops and additional time for independent study of the microscope slides (400 unknown slides with brief history, answer choices, correct diagnosis and discussion will be available). Extensive syllabus material will also be provided. The course will be held at Kensington Town Hall, London on 7–10 July 1985. Further information may be obtained from Dr Martin M. Black, Dowling Skin Unit, St Thomas' Hospital, London SE1 7EH, England.

Psoriasis Symposium, Stanford, U.S.A., July 1985

Stanford University's Department of Dermatology is presenting the Fourth International Symposium on Psoriasis, to be held 7–12 July 1985 at Stanford University. Significant advances in psoriasis research and therapy will be emphasized. For further information please contact: Paul H. Jacobs, M.D., Dept. of Dermatology, Stanford University Medical Center, Stanford, CA 94305, U.S.A.

THE ACCEPTABLE FACE OF COAL TAR



ACCEPTABLE TO YOU

In chronic psoriasis, Alphosyl offers the effectiveness of coal tar with its direct antiproliferative action on epidermal cells, together with the benefits of allantoin which helps remove plaque scales.

Alphosyl, available as a Cream, Lotion and Shampoo (Alphosyl Application PC) is therefore the ideal maintenance therapy for most types of chronic psoriasis.

ACCEPTABLE TO PATIENTS

Unlike crude coal tar preparations, Alphosyl is easy to apply, has an unobtrusive smell, and is formulated to minimise staining.

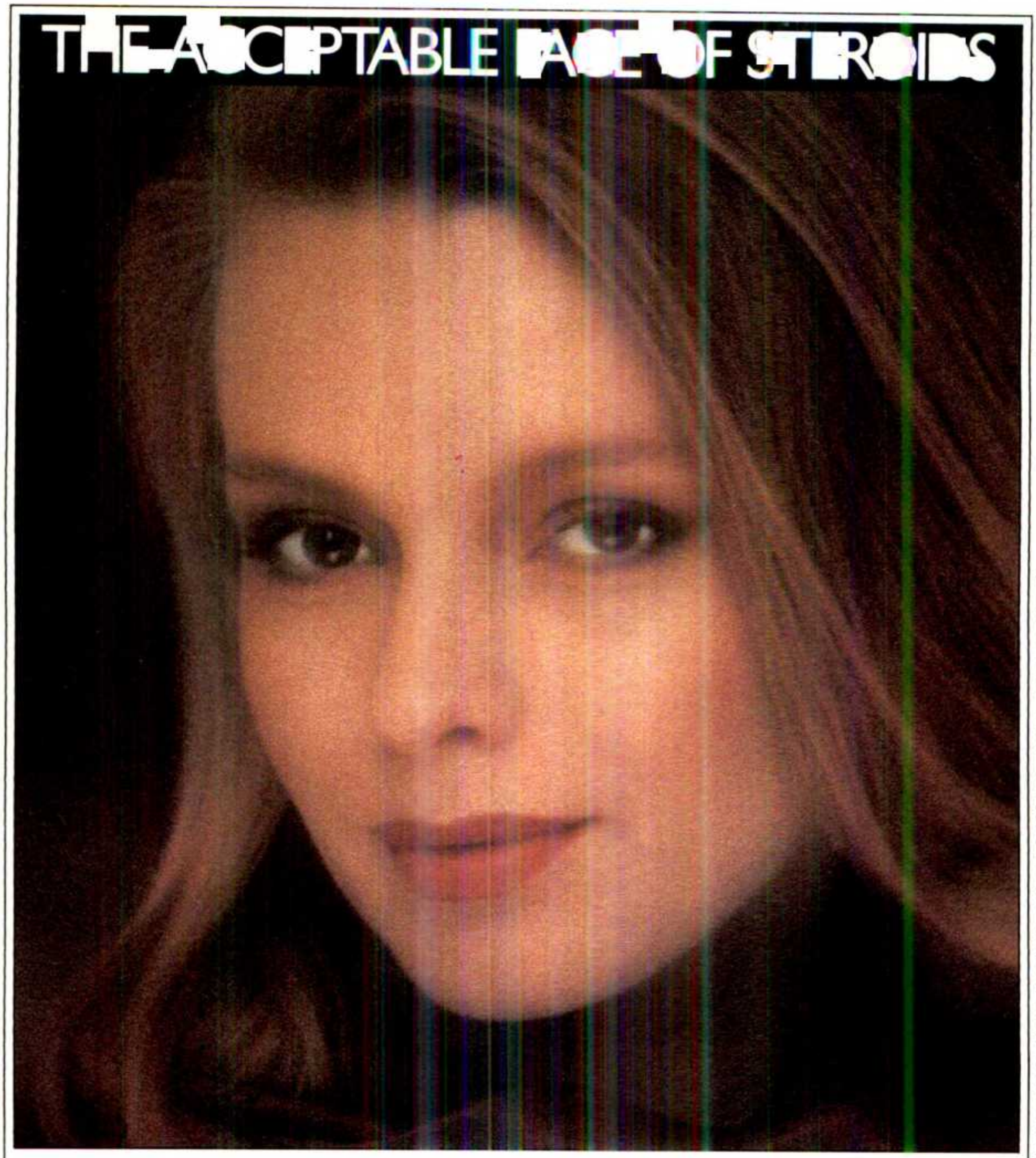
Additionally, unlike dithranol, Alphosyl will not damage healthy skin. This combination of effectiveness and cosmetic acceptability encourages patient compliance and simplifies the management of chronic psoriatic conditions.

ALPHOSYL CREAM AND LOTION

(coal tar 5%, allantoin 2%)

FULLER FREEDOM FROM PSORIASIS

Presentation LOTION AND CREAM. Containing refined alcoholic extract of coal tar 5%, allantoin 2% in a greaseless vanishing-cream base. The lotion is a light free-flowing emulsion and the cream is smooth and homogeneous, light tan in colour. **Uses** Dermatological stimulant for the treatment of psoriasis. **Dosage and administration** Apply liberally two to four times daily, massage well into the affected areas. Occlusive dressings are unnecessary. **Contra-indications, warnings etc.** For external use only. Discontinue if irritation occurs or in cases of sensitivity to coal tar. **Pharmaceutical Precautions** Do not refrigerate. Store at room temperature. **Legal category P. Package quantities/Basic NHS Cost** ALPHOSYL LOTION. Bottle of 250ml., £2.31. ALPHOSYL CREAM. Tube of 75g., £1.59. **Further information** Alphosyl Lotion is the basic treatment for psoriasis of the body and scalp. Alphosyl Cream is designed especially for lubrication in the intertriginous areas. **Product Licence Nos.** Alphosyl Lotion 0036/5008. Alphosyl Cream 0036/5006. Further information is available on request. Stafford-Miller Ltd., Professional Relations Division, Hatfield, Herts. AL10 0NZ.



THE ACCEPTABLE FACE OF STEROIDS

ACCEPTABLE TO YOU

Alphosyl HC contains low dose hydrocortisone, coal tar and allantoin for the treatment of mild to moderate psoriasis. It rapidly alleviates unsightly inflammation and avoids the need for potent steroids. Alphosyl HC can be used when transferring patients from more potent therapy.

ACCEPTABLE TO PATIENTS

Unlike many alternative treatments, Alphosyl HC has an unobtrusive smell and is formulated in a vanishing cream base to minimise staining.

Its combination of effectiveness and cosmetic acceptability encourages patients to comply with your prescription.

ALPHOSYL HC

(coal tar 5%, allantoin 2%, hydrocortisone 0.5%)

FULLER FREEDOM FROM PSORIASIS

Presentation A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone PhEur. 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogeneous and is light tan in colour. **Uses** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage and administration** Apply two to four times daily, massaging well into the affected areas. **Contra-indications, warnings etc.** In infants long-term continuous therapy should be avoided. Adrenal suppression can occur even without occlusion. Topical administration of corticosteroids to pregnant animals can cause abnormality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. Do not refrigerate. **Legal category POM. Package quantities** Tubes of 30g. and 45g. **Basic NHS cost** £1.73 for 30g., £2.46 for 45g. **Further information** Alphosyl HC cream is intended for the treatment of the acute stages of psoriasis when the basic Alphosyl action is enhanced by the addition of hydrocortisone. **Product Licence No.** 0036/0026. Further information is available on [illegible]

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a,b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

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British Journal of Dermatology

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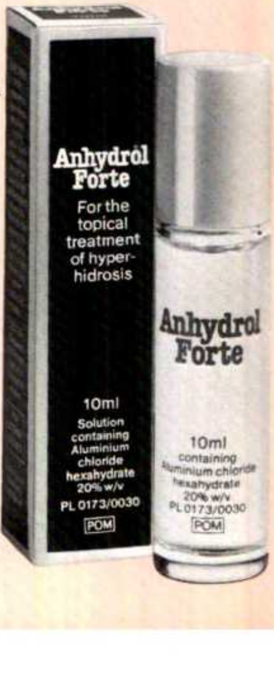
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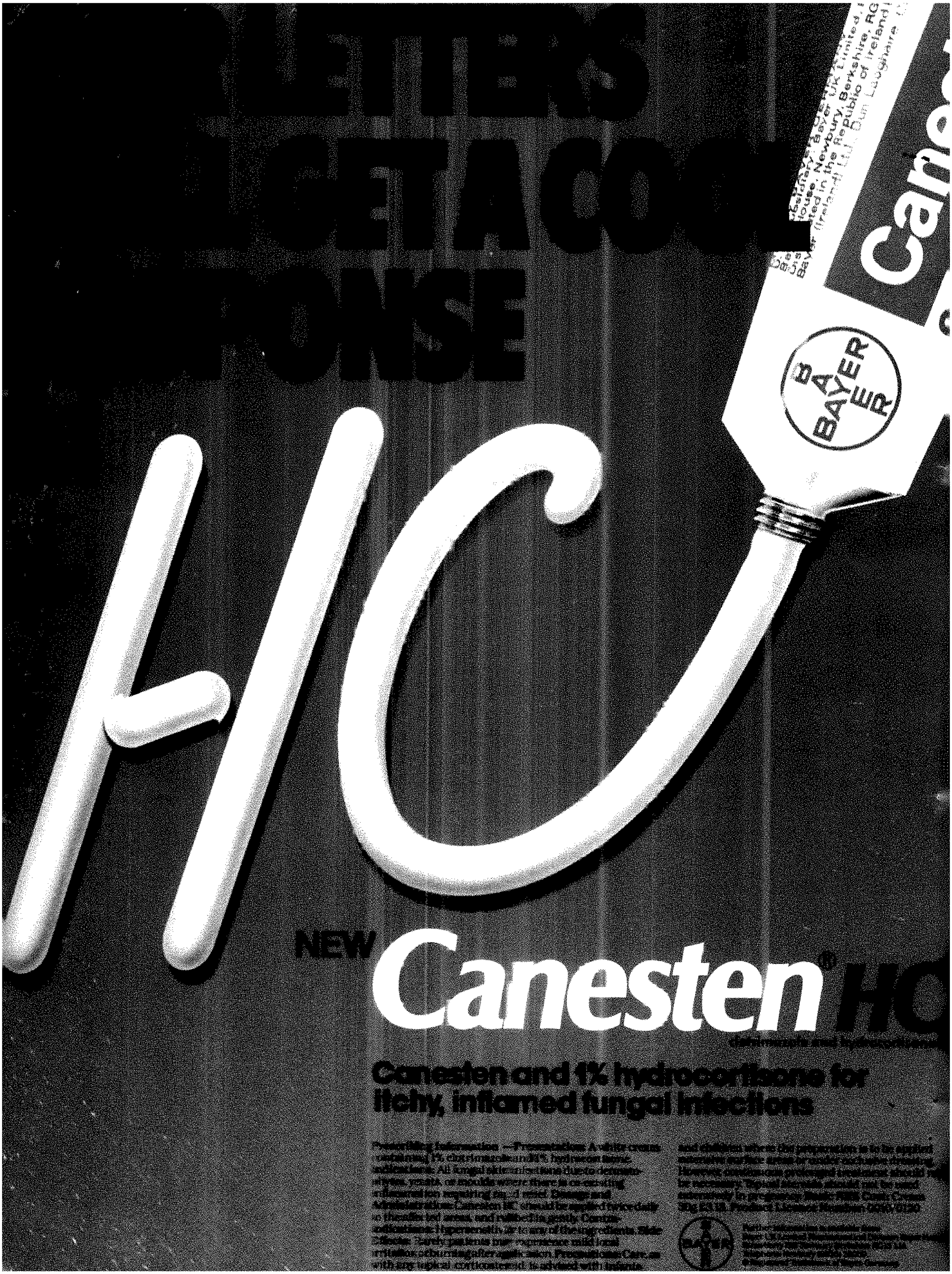
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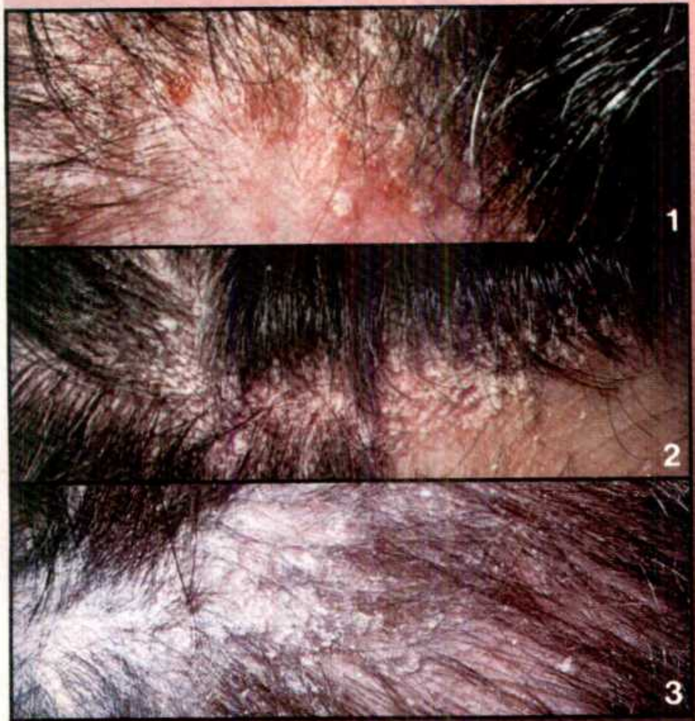

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Coat Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	91p
	150ml	£1.34
	350ml	£2.37
	1 litre	£6.18
Polytar Plus	150ml	£1.43
	350ml	£2.80

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

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Legal category: P.
Package quantities: Bottles containing 18 ml with brush fitted in cap.
Further information: Nil.
Product licence number: 0118/5010.
Basic NHS price: £0.57 per 18 ml bottle.

Reference

1. Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; 214: 657-63.

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Fry L. Br Med J 1982; 284:1093—109

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ide effects
ith all topical corticosteroids local atrophic changes may ossibly occur following prolonged and intensive treatment. lso, prolonged use of large amounts or treatment of xtensive areas may produce the features of hypercorticism. his is more likely to occur in infants and children, and with cclusion. In infants, the napkin may act as an occlusive essing.
rovided the weekly dosage of Dermovate is less than 50 rams in adults, any pituitary-adrenal suppression is likely to be ansient with a rapid return to normal values once the short ourse of steroid therapy has ceased. The same applies to ildren given proportionate dosage.
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Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.
Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

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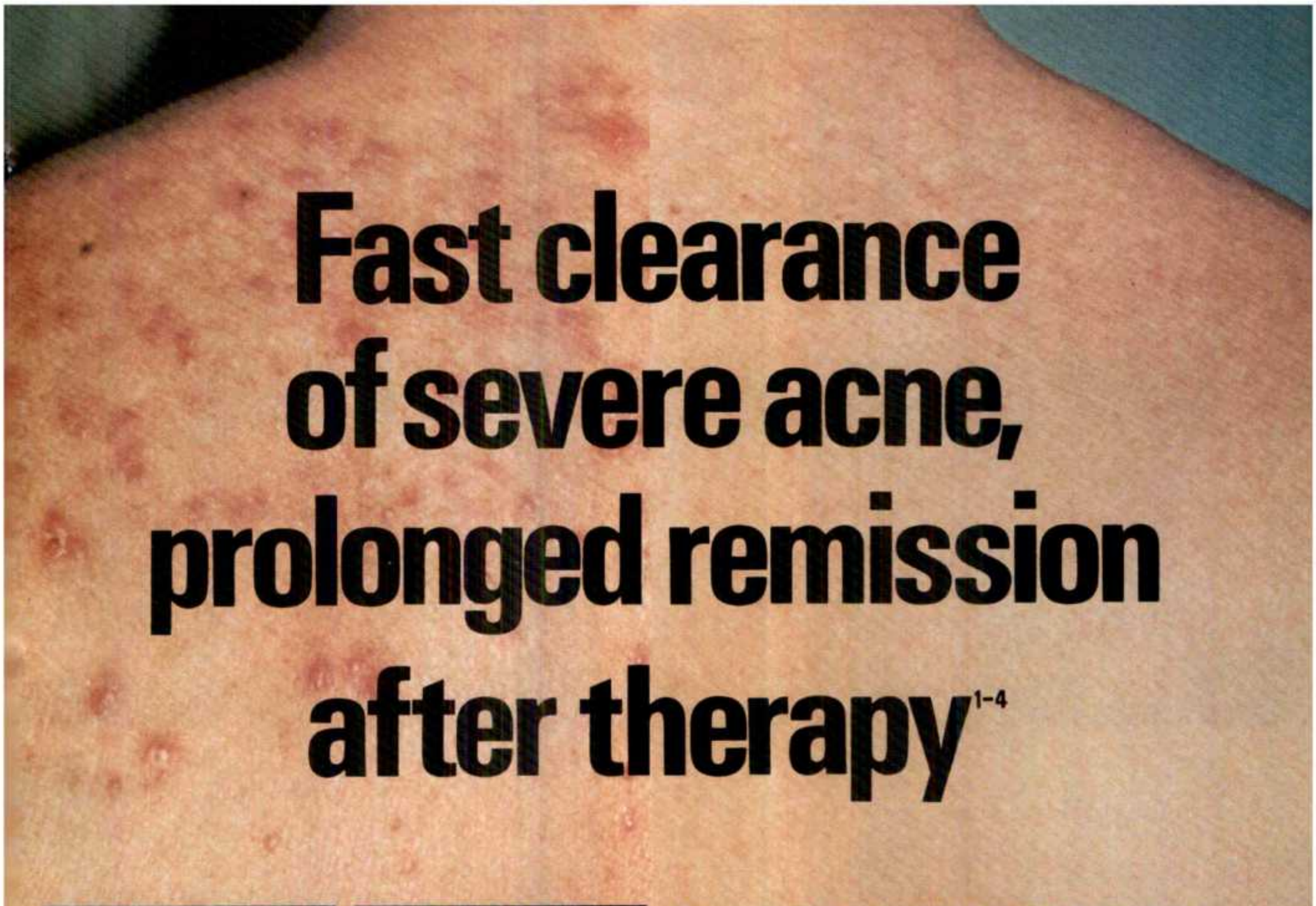

Fast clearance of severe acne, prolonged remission after therapy[1-4]

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"The aim of therapy is not only to produce clearing of active disease, but also to maintain this clearing indefinitely."[5]

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Contra-indications Pregnancy (Roaccutane is teratogenic—see 'Precautions') and breast-feeding. Hepatic or renal impairment.

Precautions The following precautions must be observed in women of childbearing potential.

1. Patients should be given detailed explanation of the risks.
2. Pregnancy should be excluded before therapy is instituted.
3. Effective contraception must be ensured for one month before treatment, during treatment, and for one month after treatment ends.

Use of patient information leaflets is strongly recommended. Monitor liver function and serum lipids (fasting value) regularly.

Side-effects Mainly mucosal dryness and fissures; facial dermatitis; desquamation, epidermal fragility; conjunctivitis; arthralgia/myalgia; increased hair loss (uncommon and reversible); possible elevation of liver enzymes and serum triglycerides. Benign intracranial hypertension has been reported, particularly in association with concomitant antibiotics.

Availability Hospitals and hospital clinics.

Presentations and Basic NHS Cost
Roaccutane capsules 5mg × 100 £33.56.
Roaccutane capsules 20mg × 100 £96.73.

Product Licence Numbers 0031/0158 (capsules 5mg), 0031/0160 (capsules 20mg).

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References

1. In: "Retinoids: Advances in Basic Research and Therapy", Eds. Orfanos, C.E. *et al.*, Springer-Verlag, Berlin, 1981, p.219.
2. Brit.J.Derm., 1983, *108*, 333.
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4. J.Amer.Acad.Derm., 1982, *6*, 766.
5. J.Amer.Acad.Derm., 1983, *9*, 629.

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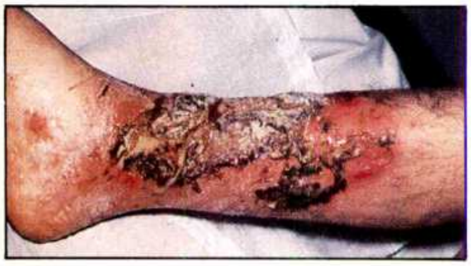

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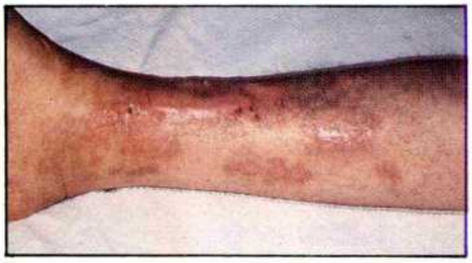

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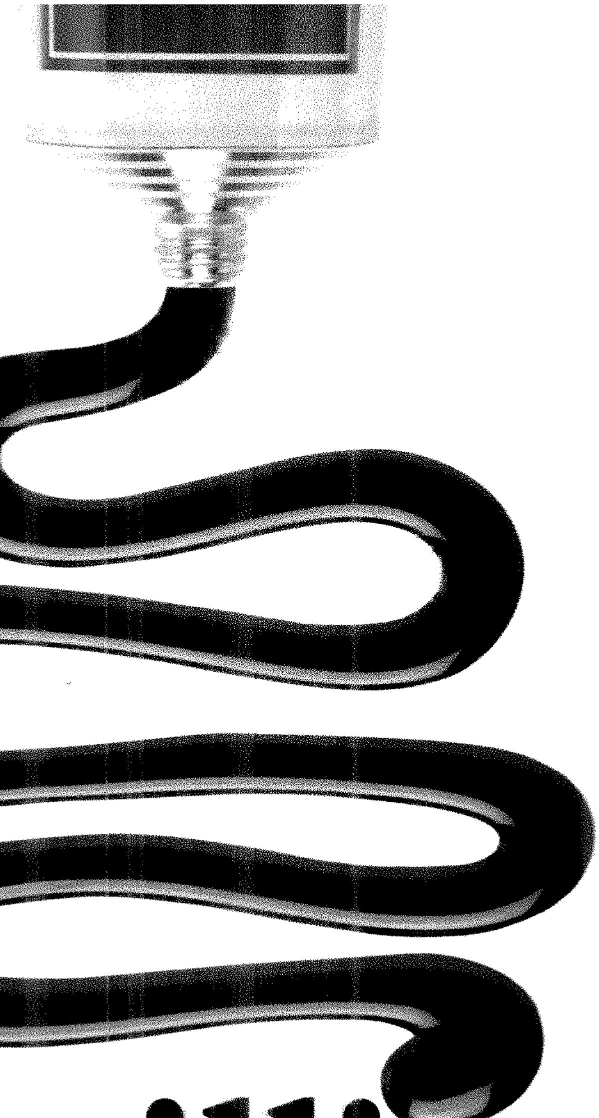

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British Journal of Dermatology (1984) **110**, 637–641.

Clinical and Laboratory Investigations

Early cellular reactions induced by dinitrochlorobenzene in sensitized human skin

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SUMMARY

Serial biopsies during the first 24 hours after dinitrochlorobenzene (DNCB) challenge in fifteen sensitized patients have shown that DNCB associates with Langerhans cells within 1 hour of application, and has reached the dermis around the appendages by 6 hours.

It is generally accepted that the epidermal Langerhans cell plays a central rôle in the contact hypersensitivity reaction. Wolff and Stingl (1983) have reviewed the evidence both for its involvement in antigen presentation in the afferent limb of the response, and later as a target cell, in the efferent limb. The Langerhans cell might thus be an integral part of the putative 'skin-associated lymphoid tissue' (SALT) of Streilein (1983).

We have studied the challenge reactions in humans previously sensitized to 2,4-dinitrochlorobenzene (DNCB) to clarify further the cellular responses in contact hypersensitivity, and to study the movement of antigen in the skin. We report our findings during the first 24 hours after challenge.

Fifteen patients have been studied (fourteen male and one female) with an age range of 45–69 years. Each was sensitized with 100 μg DNCB on an occlusive patch test applied to forearm skin, and challenged at least 2 weeks later with up to three 40 μg DNCB patch tests on the opposite forearm. Ellipse biopsies were taken from unchallenged skin in all cases, and from patch test sites at 1, 3 or 6 hours (no clinical reaction), 12 hours (erythema only) or 24 hours after application (erythema, oedema and occasional vesiculation).

Each biospy was examined by indirect immunofluorescence and indirect immunoperoxidase methods, with appropriate controls, in 5 μm cryostat sections using the antibodies shown in Table 1, and by routine electron microscopy. Forty-one biopsies have so far been studied, and the results are summarized in Table 2.

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TABLE 1. Techniques used to study the cellular infiltrate

Method	Antibody		Specificity
Indirect immunofluorescence	Anti-DNCB (I.C.I.)	Polyclonal	DNCB
Indirect immunoperoxidase	Anti-DNCB (I.C.I.)	Polyclonal	DNCB
	OKT6 (Ortho)	Monoclonal	Epidermal Langerhans cells
	OKT4 (Ortho)	Monoclonal	Helper T lymphocytes
	Leu 3a (Becton Dickinson)	Monoclonal	
	OKT8 (Ortho)	Monoclonal	Suppressor T lymphocytes
	Leu 2a (Becton Dickinson)	Monoclonal	

DNCB penetrates the epidermis very rapidly and associates with epidermal cells, both keratinocytes and Langerhans cells, within 1 hour of application. It is localized in the sweat glands and deep levels of the pilosebaceous follicles by 3 hours, and at 6 hours large, dendritic DNCB-positive cells appear in the papillary and reticular dermis, particularly around these appendages (Fig. 1).

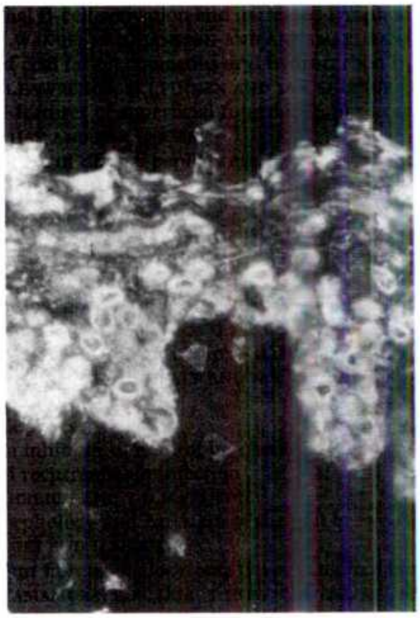

FIGURE 1.

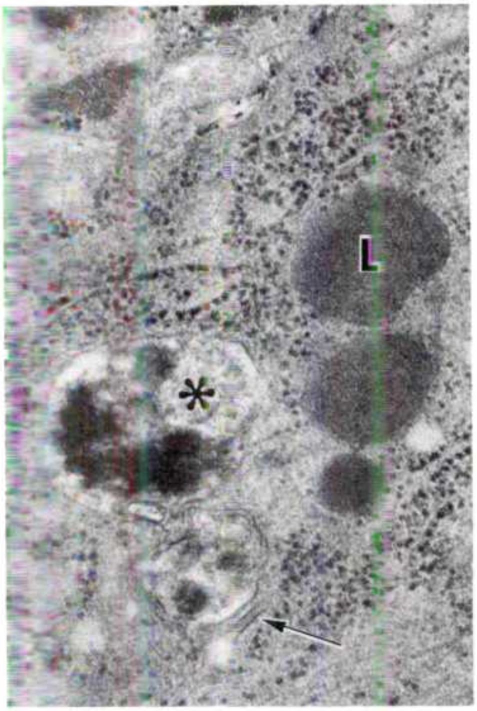

FIGURE 2.

FIGURE 1. Immunofluorescence preparation showing DNCB-positive epidermal cells, and dendritic DNCB-positive cells in dermis (× 175).

FIGURE 2. One hour after the application of DNCB a portion of epidermal Langerhans cell contains characteristic granules (arrow) together with dense, lysosome-like inclusions (L) and phagocytic vacuoles (*) (× 44,000).

TABLE 2. Summary of cellular changes

Time (h)	Anti-DNCB	OKT6	OKT4/Leu 3a	OKT8/Leu 2a	E.M.
0 (unchallenged)	Stratum corneum & collagen + (non-specific)	Suprabasal epidermal cells & dendritic network. Sparse dermal cells	Sparse dermal cells	Sparse dermal cells	
1	Upper epidermal cell membranes +	Normal epidermis ↑ Dermal cells	↑ Dermal cells around appendages	↑ Dermal cells	Langerhans cells contain dense inclusions (? lysosomes)
3	Many epidermal cells +. Sweat gland & hair follicle +	Normal epidermis. Few dermal cells	Normal pattern	Normal pattern	Normal epidermis. Langerhans-like cells in dermis, no dense inclusions
6	Large, dendritic DNCB-positive cells in papillary dermis, around appendages	Normal epidermis Many peri-appendageal dermal cells	↑ Epidermal & dermal cells	↑ Epidermal & dermal cells	Normal epidermis ↑ Langerhans-like cells in dermis. ↑ Lymphocytes around appendages
12	Epidermal cell cytoplasm +. Fewer dermal cells +	↑ Epidermal cells, loss of dendrites. Fewer dermal cells	↑ Upper dermal cells, around appendages	↓ Dermal cells	↑ Langerhans cells & Langerhans-like cells in epidermis, many damaged. Few Langerhans-like cells & lymphocytes apposed in dermis
24	Band IF at dermo-epidermal junction	↓ Epidermal cells ↑ Upper dermal cells	↑ Upper dermal cells, invading epidermis	↓ Dermal cells	↓ Epidermal Langerhans cells. Few apposed to lymphocytes. Langerhans-like cells in dermis. Histiocyte infiltrate

Epidermal Langerhans cells and dermal Langerhans-like cells (i.e. with the characteristics of Langerhans cells apart from Birbeck granules) show early activity, containing dense inclusions with the appearance of lysosomes in their cytoplasm at 1 hour (Fig. 2). OKT6-positive cells, which are normally scanty in the dermis, are numerous around skin appendages at 6 hours (Fig. 3), distributed in a similar fashion to the dendritic DNCB-positive cells. At the ultrastructural level, increased numbers of dermal Langerhans-like cells are present. Although epidermal Langerhans cell numbers are increased at 12 hours, there is electron and light microscopic evidence of cell damage, with vacuolation of Langerhans cell bodies and loss of the normal dendritic pattern. Langerhans cell numbers in the epidermis decrease by 24 hours, as dead and damaged cells are removed by macrophages.

In contrast to Silberberg, Baer and Rosenthal (1976), we found no evidence of Langerhans cell–T lymphocyte apposition in the skin before 12 hours, and very few cells were apposed at 12 or 24 hours. Our findings suggest that DNCB may be carried to the draining lymph nodes by Langerhans cells, to stimulate a lymphocytic reaction. The lymphocytes would then migrate to the challenge area with resultant cellular damage and invasion by macrophages.

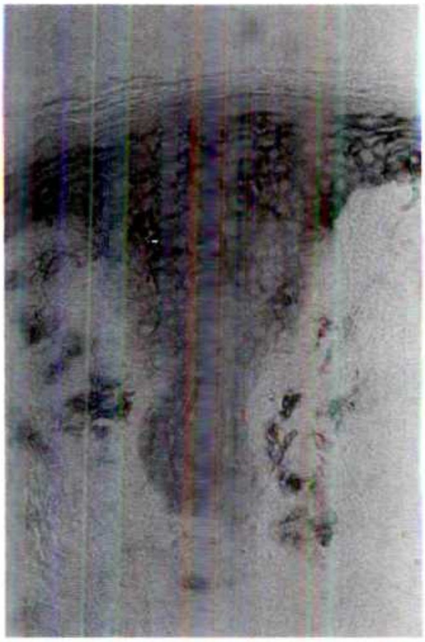

FIGURE 3. Immunoperoxidase preparation showing peri-appendageal OKT6-positive cells (× 175).

The observations that DNCB rapidly penetrates to the depths of the skin appendages and that the subsequent movements of the Langerhans cells and lymphocytes are focussed on the hair follicles and sweat ducts support the view of McCallum (1957) that these structures are important in the initiation of the contact dermatitis reaction.

We are proceeding to immunoelectron microscopic methods to identify DNCB and the cell types with which it interacts, and animal models to study the cellular reactions in skin and draining lymph nodes following challenge.

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Essential fatty acids in the plasma phospholipids of patients with atopic eczema

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SUMMARY

We have measured all the essential fatty acids (EFA) in plasma phospholipids in forty-one adults with atopic eczema and fifty normal controls. The major dietary n-6 EFA, linoleic acid, was significantly elevated, but all its metabolites, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6 were significantly reduced. The major dietary n-3 EFA, alpha-linolenic acid, was also elevated, though not significantly, while all its metabolites were also significantly reduced. These observations suggest that atopic eczema is associated not with any defect of EFA intake, but with abnormal metabolism, possibly involving the enzyme delta-6-desaturase. Treatment with oral evening primrose oil produced partial correction of the n-6 EFA abnormality, but had no effect on the n-3 EFAs.

Hansen (1937) reported that essential fatty acid (EFA) levels were low in the blood of patients with atopic eczema, and we have recently shown in two separate double-blind controlled trials that large oral doses of evening primrose oil, which contains a high concentration of EFA, produce a significant clinical improvement in patients with atopic eczema (Lovell, Burton & Horrobin, 1981; Wright & Burton, 1982). In a preliminary report (Manku *et al.*, 1982) we described the concentrations of some of the EFA in the plasma phospholipids of the patients in our second study, but no control values were available from our laboratory at that time. We have now reanalysed these samples, together with a full characterization of the remaining EFAs, and we have compared the results with matched control subjects free of atopy.

METHODS

Nomenclature of EFAs

There are two series of EFAs, the n-6 derived from linoleic acid and the n-3 derived from alpha-linolenic acid (Mead & Fulco, 1976; Kunau & Holman, 1977). These pathways are shown

TABLE 1. The pathways by which the n-6 and n-3 EFAs are metabolized

	n-6	n-3	
Linoleic	18:2	18:3	alpha-linolenic
delta-6-desaturase	↓	↓	
Gamma-linolenic	18:3	18:4	
elongation	↓	↓	
Dihomogammalinolenic	20:3	20:4	
delta-5-desaturase	↓	↓	
Arachidonic acid	20:4	20:5	eicosapentaenoic
elongation	↓	↓	
Adrenic	22:4	22:5	
delta-4-desaturase	↓	↓	
	22:5	22:6	docosahexaenoic

in Table 1. Each pathway involves alternating desaturation and elongation and it is commonly believed, though not certain, that both n-6 and n-3 EFAs are metabolized by the same enzymes.

The elongation steps are much more rapid than the desaturations, making the desaturations rate-limiting. The enzyme sequence metabolizes n-3 acids preferentially and as a result levels of 18:3 are low and 18:4 and 20:4 are undetectable except in trace amounts in human plasma. The n-6 acids are all normally readily detectable in human plasma, although 18:3n-6 is present at low levels because it appears to be formed relatively slowly from 18:2 and converted rapidly to 20:3 (Bernert & Sprecher, 1975).

Patients and samples

Adult patients with atopic eczema (equal numbers of each sex, mean age 24 years) were entered into a double-blind, placebo-controlled, crossover trial of evening primrose oil in the treatment of atopic eczema. All patients had clinically typical atopic dermatitis with a history beginning in childhood, together with a personal and/or family history of other atopic disorders. The evening primrose oil used was Efamol®, the seed oil from a specially bred strain of *Oenothera biennis* which yields oil of a constant composition. Efamol® contains 72% of 18:2n-6 and 9% of 18:3n-6. Three dose levels were used, 2, 4, and 6 g/day. Patients were assigned to receive placebo first or Efamol® first for 12 weeks, then the alternative treatment for a further 12 weeks. The clinical results of the trial have been reported elsewhere: there was a significant dose-related improvement in clinical condition (Wright & Burton, 1982).

There were duplicate samples for some but not all patients, since some of the samples were fully used in the previous analysis. For the current analysis forty-one pairs of samples were available: these pairs were the last baseline sample before entering the trial, and the last sample taken at the end of the Efamol® treatment period. Sixteen were from patients on 2 g, 13 from those on 4 g and 12 from those on 6 g/day. The samples were taken into EDTA-treated tubes, centrifuged and then deep frozen until analysis. Calculations were initially performed on male and female samples separately but as there were no significant differences between the two, all the analyses reported in this paper are for both sexes together.

Controls were fifty university students (thirty-two males, eighteen females, mean age 20 years). Again there were no significant differences in plasma EFA levels between the sexes. All controls were free of acute or chronic illness, denied taking either medicinal or recreational drugs and had no personal or family history of atopy.

Plasma samples (1 ml) were extracted with chloroform:methanol (2:1). The extract was filtered through sodium sulphate, evaporated to dryness, and taken up in 0·5 ml chloroform: methanol. The lipid fractions were separated by thin-layer chromatography on silica gel plates. The phospholipid fraction, which seems to reflect essential fatty acid changes most sensitively, was methylated using boron trifluoride-methanol. The resulting methyl esters of the fatty acids were separated and measured using a Hewlett Packard 5880 gas chromatograph with a 6-foot column packed with 10% silar on chromosorb WAW 106/230. The carrier gas was helium (30 ml/min). Oven temperature was programmed to rise from 165°C to 190°C at 2°C/min. Detector temperature was 220°C and injector temperature 200°C. Retention times and peak areas were automatically computed by a Hewlett Packard Level 4 integrator. Peaks were identified by comparison with standard fatty acid methyl esters from Nuchek Prep. Inc. (Elysian, Minnesota, U.S.A.).

RESULTS

The results are shown in Tables 2 and 3. All fatty acid levels in the normal controls were very close to those reported in a previous large study performed by Holman, Smythe & Johnson (1979). For example, Holman's figures for 18:2n-6, 20:3n-6, and 20:4n-6 were 20·2%, 3·4%, and 11·5%, whereas ours were 21·4%, 3·1%, and 11·4% respectively. In the atopic patients, linoleic acid, the main dietary n-6 EFA, was highly significantly elevated. Gamma-linolenic acid was undetectable in all but three of the samples from patients. All subsequent n-6 metabolites

TABLE 2. Fatty acid levels in plasma phospholipids in fifty control individuals, and in forty-one patients with atopic eczema before and after treatment with 18:3n-6. Results are expressed as means ± s.d. Statistical analysis was by Student's *t*-test

Fatty acid	Control	Eczema baseline	Eczema post-Efamol®
16:0	25·81 ± 1·69	30·14 ± 1·86***	30·00 ± 2·17
18:0	11·61 ± 1·32	13·48 ± 1·78**	13·56 ± 2·52
18:1n-9	13·53 ± 2·21	14·11 ± 1·31	14·56 ± 1·27
18:2n-6	21·45 ± 2·81	24·50 ± 3·42*	24·38 ± 3·73
18:3n-6	0·16 ± 0·11	—	—
20:3n-6	3·06 ± 1·60	2·63 ± 0·52**	3·13 ± 0·58‡
20:4n-6	11·36 ± 1·67	6·75 ± 1·12***	7·45 ± 1·46†
22:4n-6	0·73 ± 0·26	0·38 ± 0·24***	0·33 ± 0·26
22:5n-6	1·12 ± 0·67	0·13 ± 0·09***	0·11 ± 0·09
18:3n-3	0·27 ± 0·53	0·36 ± 0·17	0·39 ± 0·34
20:5n-3	1·01 ± 0·36	0·75 ± 0·57*	0·72 ± 0·76
22:5n-3	0·93 ± 0·27	0·74 ± 0·19**	0·70 ± 0·24
22:6n-3	3·54 ± 0·89	2·43 ± 0·62***	2·36 ± 1·01

* Different from control at $P < 0·01$; ** Different from control at $P < 0·001$; *** Different from control at $P < 0·0001$ or better.

† Different from baseline at $P < 0·01$; ‡ Different from baseline at $P < 0·001$.

TABLE 3. In Table 2 all three dose levels were grouped together. This table shows the post-Efamol® increases in levels of 20:3n-6 and 20:4n-6 with respect to dose

	20:3n-6	20:4n-6
2 g/day Efamol®	0·08	0·88
4 g/day Efamol®	0·37	0·36
8 g/day Efamol®	1·20	0·83

were highly significantly reduced. The picture in the n-3 pathway was similar. 18:3n-3 was elevated though not significantly so. All the n-3 metabolites, however, were highly significantly reduced. Possibly in compensation for the low levels of EFAs, the two major saturated fats in plasma phospholipids, palmitic (16:0) and stearic (18:0) were highly significantly elevated. The mono-unsaturated oleic acid (18:1n-9) was slightly but not significantly raised.

Treatment with Efamol® produced highly significant elevations in 20:3n-6 and 20:4n-6. DGLA became normal, but arachidonic acid remained well below normal even after 3 months' treatment. The C-22 n-6 fatty acids were unchanged as were all the n-3 EFAs.

Table 2 groups all the post-Efamol® dose groups together. Table 3 shows some evidence of a dose-response effect which is clear with DGLA but not with arachidonic acid.

DISCUSSION

The results confirm Hansen's 1937 finding of an elevated linoleic/arachidonic acid ratio and our earlier report of reduced 18:3n-6, 20:3n-6, and 20:4n-6 in comparison with literature controls (Manku *et al.*, 1982). The use of matched controls specifically selected to be free of atopy, and the measurement of the levels of all the EFAs has now allowed a more comprehensive picture to emerge.

It seems that there is no defect of dietary intake or absorption of EFAs in the atopic eczema patients. The levels of the two main dietary EFAs were actually somewhat above control values. In contrast, all the metabolites in both the n-6 and n-3 series were highly significantly reduced. If the same enzyme sequence is involved in metabolism of both n-6 and n-3 EFAs, the most likely explanation for these observations is that the functioning of the first enzyme in the sequence, the delta-6-desaturase, is defective. There are many possible reasons for this, including a minor abnormality in the protein structure of the enzyme, or an abnormality of cofactors. This question can be resolved only by further research.

Figures 1 and 2 express the changes in fatty acid levels as percentages of those found in the controls. This suggests that the other two desaturases may possibly be affected. The fall in the products of desaturation is in each case greater than the fall in the substrates, at least as far as the n-6 EFAs are concerned. However, the desaturases are rate-limiting (Bernert and Sprecher, 1975), and the pattern observed may simply be a consequence of reduced inflow into a system in which elongation is very rapid and desaturation rather slow.

The differences from normal are so striking, so consistent among the EFAs of a particular type, and so similar in the two groups of EFAs, that there can be no doubt that there is an abnormality of EFA metabolism in patients with atopic eczema. Whether or not this

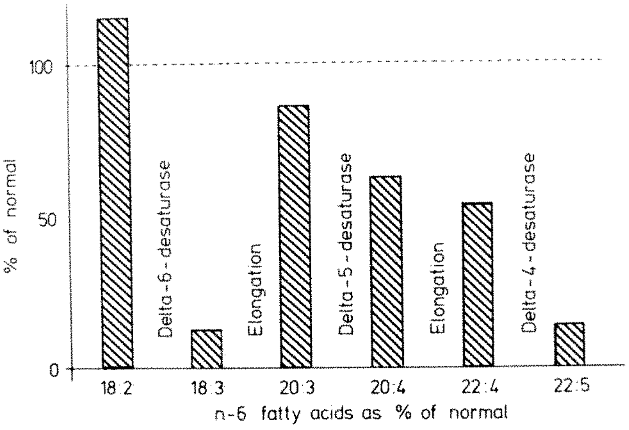

FIGURE 1. n-6 Fatty acids as percentage of normal.

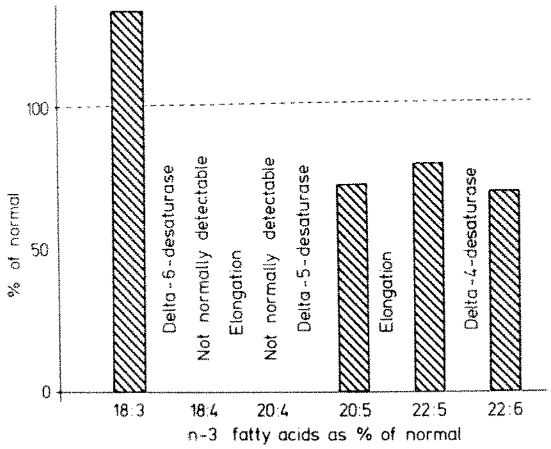

FIGURE 2. n-3 Fatty acids as percentage of normal.

abnormality plays a causative role requires further evaluation, but it is interesting that significant clinical improvement followed the administration of an oil containing 18:3n-6, which would bypass the first step in the n-6 pathway. It is evident that full correction of the n-6 levels throughout the body, especially with regard to the C-22 acids, may require prolonged treatment with 18:3n-6. Correction of the n-3 acids will require the administration of 20:5n-3 which is to be found in marine oils. Only when the clinical response to full correction of both the n-6 and n-3 abnormalities is observed will it be possible to make a definitive statement about the role of the EFA changes in the clinical expression of atopy.

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Small intestinal permeability to sugars in patients with atopic eczema

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SUMMARY

Absorption of lactulose and mannitol was measured in eleven patients with atopic eczema and lactulose/mannitol excretion ratios were calculated. Mean lactulose absorption was increased in the patients with eczema and their excretion ratios were higher than those of controls. There was no correlation between either eczema extent or severity and the excretion ratio. We conclude that small intestinal passive permeability is increased in some patients with atopic eczema.

It has been suggested that food allergy may play a role in the pathogenesis of atopic eczema (Atherton, 1981). It is possible that the small intestine in patients with atopic eczema might be permeable to potential dietary allergens which would be excluded in non-eczematous subjects; there is some evidence that this might be so (Paganelli *et al.*, 1979; Jackson *et al.*, 1981).

Passive permeability of the small intestine can be studied by measuring the simultaneous absorption of mannitol (a polyhydric alcohol, mol. wt. 180) and lactulose (a disaccharide, mol. wt. 342) and the ratio of mannitol to lactulose absorption provides an index of small intestinal passive permeability (Ukabam, Clamp & Cooper, 1983). The aim of this study was to measure passive permeability in a group of randomly selected patients with atopic eczema of varying severity.

METHODS

Eleven adults with atopic eczema (eight males; three females) aged 18–47 years (mean 31 years) were studied. None had symptoms of gastrointestinal disease, but two had a history of food allergy. Twenty-five normal adults (nine males; sixteen females) aged 19–65 years (mean 34 years) without any evidence of gastrointestinal or skin disease served as controls. The extent of skin involvement in the patients with eczema was mild (0–25%) in five patients, moderate

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(26–50%) in one patient, moderately severe (51–75%) in two patients and severe (76–100%) in three patients. A severity index which has been previously described (Clendenning *et al.*, 1973) was calculated for each patient scoring 0 to 4 for each of eight signs (erythema, lichenification, oedema, pigmentation, scaling, vesiculation, excoriation and crusting) and two symptoms (pruritus and lack of sleep).

All subjects gave informed consent to the permeability studies and after an overnight fast, each subject drank 0·5 g mannitol and 10 g lactulose in 100 ml water. All urine passed was collected over 6 hours and an aliquot was stored at −10°C until analysis. The quantities of lactulose and mannitol in the urine samples were measured by a gas–liquid chromatographic method, using inositol as internal standard, which has been previously described (Ukabam *et al.*, 1983). The lactulose and mannitol quantities were expressed as percentages of the oral dose and lactulose/mannitol excretion ratios (LMER) were calculated.

Mannitol and lactulose excretion were compared using Student's *t*-test. Lactulose/mannitol excretion ratios were compared by the Wilcoxon rank sum test and correlations were studied with Spearman's correlation coefficient.

RESULTS

All subjects tolerated the oral sugar loads without ill effects.

Mannitol. There was no significant difference between mean mannitol excretion in the patients with eczema (mean 13 ± 5·3 s.d. %) and the controls (15·9 ± 4·4%) (Fig. 1).

Lactulose. The mean lactulose excretion in the patients with atopic eczema (0·26 ± 0·13%) was significantly higher than that of the controls (0·15 ± 0·01%) ($t = 2·8$; $P < 0·01$) (Fig. 1).

Lactulose/mannitol excretion ratios (LMER). LMER in the patients with atopic eczema, range 0·007–0·047 (median 0·019) were significantly higher than those of the controls, range

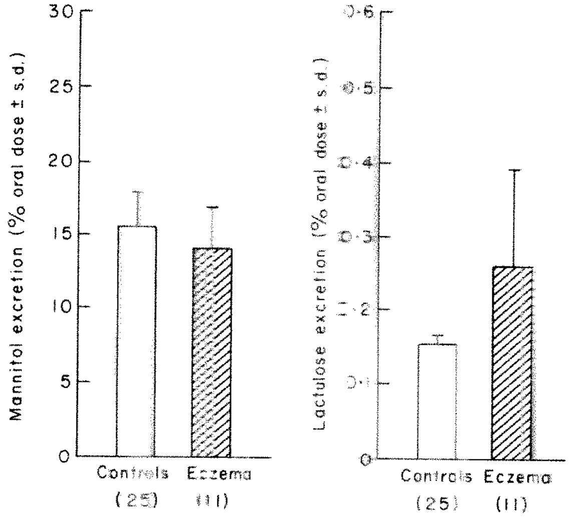

FIGURE 1. Mannitol and lactulose excretion in normal controls and in patients with atopic eczema.

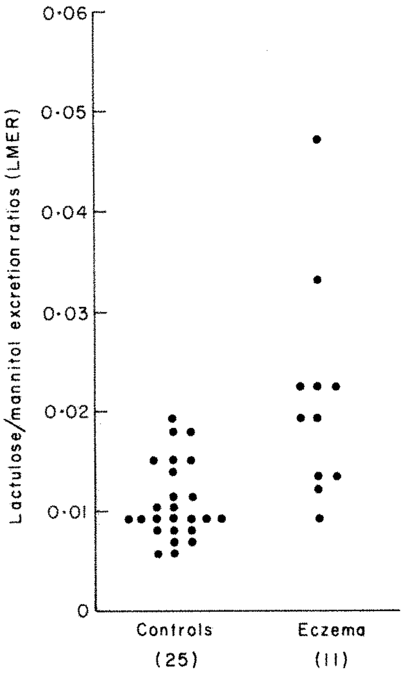

FIGURE 2. Lactulose/mannitol excretion ratios in normal controls and in patients with atopic eczema.

0·006–0·019 (median 0·009) ($P < 0·01$) (Fig. 2). Five patients with eczema had LMER outside the normal range. However, there was no correlation between LMER and the extent of skin involvement ($\Sigma D^2 + T = 194$; $P > 0·1$) or between LMER and the severity of the eczema ($\Sigma D^2 + T = 330$; $P > 0·1$) (Table 1). LMER of the two patients with food allergy were 0·013 and 0·022.

TABLE 1. LMER, extent and severity of eczema in patients with atopic eczema

Patient	Sex	Eczema extent (%)	Severity index (max. 40)	Lactulose/mannitol excretion ratio (LMER)
T.W.	M	76–100	12	0·022
P.M.	M	76–100	15	0·033
S.W.	M	76–100	8	0·013
J.M.	F	51–75	9	0·008
B.H.	M	51–75	12	0·019
L.H.	M	26–50	20	0·022
J.B.	F	0–25	10	0·019
C.B.	M	0–25	10	0·019
I.A.	M	0–25	9	0·012
W.M.	M	0–25	5	0·047
E.F.	F	0–25	2	0·022

DISCUSSION

Our results showed that five of the eleven patients had LMER outside the normal range and that as a group, the patients with atopic eczema had LMER higher than the normal controls, indicating an abnormality of passive permeability of the small intestine. The abnormalities in LMER were the result of increased lactulose absorption, indicating that the mucosa of the patients was more 'leaky' than normal (Hamilton *et al.*, 1982; Ukabam *et al.*, 1983). In contrast mannitol absorption, which reflects small intestinal surface area (Hamilton *et al.*, 1982; Ukabam *et al.*, 1983), was normal.

Although jejunal biopsy studies of children with eczema have reported minor histological abnormalities in some cases (McCalla *et al.*, 1980), there has only been one other study of passive permeability of the small intestine in patients with eczema (Jackson *et al.*, 1981). A totally different method of assessing permeability was used, with polyethylene glycol 600 and 6000 as permeability markers. However, nine of eighteen patients with eczema had evidence of increased small intestinal passive permeability.

These two studies of passive permeability provide good evidence that the small intestinal mucosa in some patients with atopic eczema is more permeable than normal. This indicates that dietary antigens could play a role in the pathogenesis of atopic eczema, but further studies are required to determine whether or not these permeability changes are a primary defect.

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Increased concentrations of immunoreactive leukotrienes in cutaneous lesions of eosinophilic cellulitis

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SUMMARY

A case of eosinophilic cellulitis is described. Tests of eosinophil function were normal. Radioimmunoassay identification of inflammatory mediators showed greatly increased concentrations of leukotrienes LTC_4/D_4 (components of slow-reacting substance of anaphylaxis) in the affected skin. These mediators may play a causal role in the inflammation seen with eosinophilic cellulitis.

Eosinophilic cellulitis, first reported by Wells (1971) and described in further detail by Wells and Smith (1979), consists of recurrent episodes of cutaneous and subcutaneous redness and swelling followed by a second phase of indolent induration which may last for several weeks. Eventually complete resolution occurs. Blood and bone marrow eosinophilia is present in the acute phase. The histopathological features of the active lesion are distinctive, and include intense dermal oedema and a heavy invasion of the affected dermis by eosinophils interspersed with histiocytes, with amorphous eosinophilic deposits called flame figures, and, in acute lesions, with eosinophil granules. Wells and Smith (1979) have drawn attention to the development of similar histological appearances as a reactive process in response to fungus infection, pemphigoid and eczema. The molecular mechanisms of eosinophilic cellulitis are unknown, but the pathological appearances are compatible with local release of potent eosinophilotactic activity, perhaps in response to a primary immunological process. Deranged eosinophil leukocyte function is also a possibility. We report here a patient with classic features of eosinophilic cellulitis in whom we have carried out preliminary studies of eosinophil function. We have also analysed tissue fluid for mediator activity and have detected increased concentrations of immunoreactive leukotriene C_4/D_4 (LTC_4/D_4) in exudate obtained on several occasions from lesional skin.

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CASE HISTORY

A.D., a 28-year-old man, presented in December 1982 with an acute eruption of giant urticaria-like lesions affecting the whole of his left arm and both thighs. This lasted 5 weeks. A similar episode affecting his left arm occurred in 1978 while he was in Germany and this was thought to be allergic contact dermatitis. He had no previous history of skin disease and did not have a history suggestive of atopy. In February 1983 he developed acute swelling affecting the dorsa of his feet which gradually extended to involve the whole of both lower limbs. Blisters, which later became haemorrhagic, developed on the swollen feet. He was afebrile and examination of other systems was normal.

There was no improvement with antihistamines, non-steroid anti-inflammatory drugs or antibiotics. A reducing course of prednisolone starting at 40 mg produced a dramatic improvement. To date no relapse has occurred.

INVESTIGATION

The total white cell count was raised at 16,200/mm^3 with 18% eosinophils (2916/mm^3), 57% neutrophils and 24% lymphocytes. The ESR was 2 mm/h. Urea, electrolytes, liver and thyroid function, serum calcium and urate were normal. VDRL, toxocara and hepatitis B antigen tests were negative. Antinuclear and rheumatoid factor, immunoglobulin, complement, immune complexes and C1 esterase inhibitor were normal. Mycological culture of skin scrapings and stool examination for ova, cysts and parasites were negative. Electrocardiogram, echocardiogram and pulmonary function tests were within normal limits.

Bone marrow examination (Professor J.W.Stewart, Middlesex Hospital, London) carried out when the patient was receiving systemic steroids, revealed myeloid hyperplasia and mild eosinophilia. Philadelphia chromosome was not detected and the neutrophil alkaline phosphatase reaction was normal.

Histology of the involved skin (Dr Russell Jones) revealed a dense dermal infiltrate of neutrophils and eosinophils extending to the subcutis with some tendency towards a perivascular arrangement but there was no evidence of a vasculitis. Eosinophilic flame figures were seen in the dermis. Direct immunofluorescence was negative.

The chemotactic response of neutrophils to *E. coli* endotoxin in the Boyden chamber was normal [mean count per high power field 63 (stimulated); 18 (unstimulated); ratio 3·5 : 1 (normal ratio 1·1 : 3·8)].

In vitro studies on the patient's eosinophils in a complement and antibody dependent schistosomula (*S. mansoni*) cytotoxicity assay (Anwar *et al.*, 1980) were comparable to eosinophils from normal individuals, as was the ability to show enhanced cytotoxicity to schistosomula by pre-incubation with histamine (10^{-7}/mol/l) and formyl methionyl leucyl phenylalanine (10^{-8}/mol/l).

Exudate was obtained from inflamed skin and from adjacent clinically normal skin by a suction bulla technique (Kobza Black *et al.*, 1977). Immunoreactive LTC_4/D_4 and LTB_4 were assayed on 0·1 ml volumes of exudate by radioimmunoassay utilising rabbit antisera against LTC_4 as described by Hayes *et al.* (1983). The immunoreactant raised against LTC_4 cross-reacts to a major degree with LTD_4. The results (Table 1) showed that immunoreactive LTC_4/D_4 was present in greatly increased amounts in involved skin recovered on two separate occasions from the same patient. It is also of interest that increased concentrations were found in exudate from clinically normal skin of the same patient. Increased levels of immunoreactive LTB_4 were also found in involved skin but not in clinically normal skin.

TABLE 1. Concentrations of immunoreactive LTC_4/D_4 and LTB_4 in exudate

Exudate	LTC_4/D_4 ng/ml	LTB_4 ng/ml
Involved skin A	4·7	0·2
Involved skin B	2·6	0·4
Uninvolved skin	1·2	0·0

A and B represent samples obtained on different occasions.

LTC_4/D_4: upper limit of normal = 0·03 ng/ml exudate.

Immunoreactive LTB_4 is undetectable in normal skin exudate.

DISCUSSION

The aetiology of eosinophilic cellulitis is not known. The erythema and oedema in the skin represent an acute inflammatory process. Whether the eosinophils which feature so prominently in this condition could play a role in producing this inflammation is not clear. There is evidence that major basic protein (MBP), a primary constituent of eosinophil granules, induces non-cytolytic release of histamine from human basophils and rat peritoneal mast cells (O'Donnell *et al.*, 1983). MBP has also been shown to be cytotoxic to many mamalian cells (Gleich *et al.*, 1979). Furthermore, large quantitites of MBP are present in the flame figures of eosinophilic cellulitis (Peters, Schroeter & Gleich, 1983). These studies suggest that in eosinophilic cellulitis the eosinophils could play a significant role in mediating inflammatory reactions.

We have demonstrated the presence of LTC_4/D_4 in the affected skin. Leukotrienes are formed by the transformation of arachidonic acid via the 5-lipoxygenase pathway (Samuelsson *et al.*, 1979) into an unstable epoxide intermediate, leukotriene A_4, which then undergoes further enzymatic conversion to LTC_4. LTD_4 is formed by the elimination of the γ-glutamyl residue of LTC_4. LTC_4 and LTD_4 are components of slow reacting substance of anaphylaxis (SRS-A), a potent mediator of inflammation (Lewis *et al.*, 1980). Camp *et al.* (1982) and Soter *et al.* (1983) have shown that intradermal injections of LTC_4 and LTD_4 in nanomolar amounts produce erythema and wealing. The identification of these pro-inflammatory mediators in the skin of eosinophilic cellulitis correlates well with the clinical manifestation of inflammation in this condition. Whether the eosinophils are the source of LTC_4/D_4 remains speculative. LTC_4 and LTD_4 have been demonstrated in horse eosinophils (Jörg *et al.*, 1982). In man LTC_4 and LTD_4 have been demonstrated in polymorphonuclear leukocytes, lung and eosinophils (Samuelsson, 1983; K.F.Austen, personal communication). Since SRS-A is mainly derived from mast cells it is conceivable that LTC_4 and LTD_4 could be released from stimulated mast cells. The eosinophil major basic protein (MBP) could evoke release from mast cells of SRS-A as well as histamine (O'Donnell *et al.*, 1983).

The finding of elevated levels of LTB_4 is also of interest. LTB_4 may evoke inflammation in human skin because of its highly potent neutrophil chemo-attractant properties (Ford-Hutchinson *et al.*, 1980; Camp *et al.*, 1984). It also possesses potent eosinophilotactic properties

(Nagy *et al.*, 1982) and could contribute to the attraction of eosinophils into the affected skin. Eosinophils as well as neutrophils can generate LTB_4 (Jörg *et al.*, 1982).

Thus eosinophils may play a significant role in the pathogenesis of eosinophilic cellulitis by providing the source of LTC_4/D_4 and LTB_4 or by stimulating mast cells (via MBP) to release these mediators of inflammation.

ACKNOWLEDGMENTS

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Inhibition of chemiluminescence in human neutrophils by dapsone

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SUMMARY

Dapsone at doses of 0·5 to 5·0 μg/ml was found to produce a dose-dependent inhibition of opsonized zymosan-induced human polymorphonuclear leukocyte (PMN) chemiluminescence (CL) *in vitro*. Simultaneous exposure of PMN to dapsone and zymosan was as effective in reducing CL as preincubation of PMN with dapsone. Preincubation of PMN with dapsone followed by washing, resulted in the loss of dapsone-mediated CL inhibition, indicating that dapsone did not permanently alter the CL-generating mechanism and that the drug had to be present to inhibit CL. Dapsone did not absorb light at the wavelength of CL and was not toxic to PMN at concentrations tested. Sodium azide, an inhibitor of myeloperoxidase-mediated CL inhibited PMN CL to the same degree as dapsone. When incubated together with PMN, dapsone and azide did not produce an additive inhibition of CL. These data suggest that inhibition of myeloperoxidase may be the mechanism by which dapsone inhibits PMN CL.

Dapsone is a sulphone compound with a variety of therapeutic uses. It is the drug of choice for the treatment of leprosy and is employed in malaria treatment and prophylaxis (Faset *et al.*, 1943). It has also been shown to be effective in reducing the intensity of certain non-infectious inflammatory diseases including dermatitis herpetiformis (Katz *et al.*, 1976), subcorneal pustular dermatosis (Sneddon & Wilkinson, 1956), leukocytoclastic vasculitis (Winkleman & DiHo, 1964), Crohn's disease (Ward & McManus, 1978), pustular psoriasis (Sneddon & Wilkinson, 1956), and erythema elevatum diutinum (Katz *et al.*, 1977).

The interactions of dapsone with inflammatory systems are not well understood. Dapsone does not inhibit complement activation (Katz *et al.*, 1976), polymorphonuclear leukocyte (PMN) phagocytosis, lysosomal enzyme release, or chemotaxis to activated serum (Esterly *et al.*, 1978; Ward, Goralnick & Bullock, 1976; Anderson *et al.*, 1981) but it has recently been shown to inhibit the chemotactic response of PMN to the synthetic peptide f-met-leu-phe (Harvath, Yancey & Katz, 1983). Mier and van den Hurk (1975) reported that very high concentrations of the drug inhibit certain hydrolytic enzymes, and recent work by Stendahl and colleagues (1978) has shown that therapeutically-attainable concentrations of dapsone inhibit the myeloperoxidase–peroxide–halide (MPO) system of human PMN *in vitro*. In this study, dapsone was found to decrease the MPO-mediated functions of lymphomacidal activity and candidacidal activity as well as direct inhibition of protein iodination by PMN.

In addition to stimulating cytocidal activity (Clark & Klebanoff, 1975), MPO has also been implicated in the phenomenon of polymorphonuclear leukocyte chemiluminescence (PMN CL) by the findings that isolated PMN MPO emits light at the same wavelength as PMN CL (Anderson, Brenzel & Lint, 1977) and PMN deficient in MPO have depressed CL (Rosen & Klebanoff, 1976). Diminished CL has also been reported in individuals with PMN defective in oxidative metabolism, in severe neutropaenia, and gram-negative septicaemia (Stevens, Winston & Van Dyke, 1978), and in the very young and the very old (Van Epps, Goodwin & Murphy, 1978), and it has become accepted as indicative of impaired PMN function. Since dapsone has been shown to inhibit PMN MPO, a compound involved in the generation of CL, we undertook to study the effect of dapsone on the generation of PMN CL in order to define further the potential mechanism of dapsone's anti-inflammatory activity.

METHODS

Dapsone (4,4′-diaminodiphenylsulphone) (Aldrich Chemical Co., Metuchen, N.J.) was dissolved in absolute ethanol at a concentration of 10·0 mg/ml. Prior to use this stock solution was diluted with Hank's Balanced Salts Solution (HBSS) to working concentrations. In all cases an appropriately diluted ethanol–HBSS control was included in the controls.

PMN isolation. Human peripheral blood PMN were purified from the blood of normal adult volunteers by a combination of dextran-sedimentation of heparinized blood followed by centrifugation of 'buffy coat' cells over lymphocyte separation medium (Litton Bionetics, Kensington, Md.). Contaminating erythrocytes were removed from this PMN-enriched preparation by treatment with hypotonic saline. The resulting preparations were uniformly greater than 95% PMN with greater than 98% viability as assessed by Trypan Blue exclusion. Before testing, PMN were suspended to a concentration of 5×10^6/ml in HBSS.

Opsonization of zymosan. Zymosan (Schwartz-Mann, Orangeburg, N.Y.) was boiled for 30 minutes, washed in HBSS, and incubated with a 1 : 2 dilution of fresh type AB human serum for 30 minutes at a concentration of 20 mg/ml. After incubation, the zymosan was separated from the serum by centrifugation, washed and resuspended in HBSS. Opsonized zymosan (2 mg/ml) was added to the PMN cultures and the production of CL was assayed as described below.

Chemiluminescence assay. PMN CL was assayed in HBSS, pH 7·4, using opsonized zymosan as the stimulant. Assays were performed in 'dark-adapted' (7 ml) plastic scintillation vials (Walter Sarstad Inc., Princeton, N.J.). CL was measured in an unrefrigerated PRIAS liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.), set in an 'out of coincidence' mode, 50% gain, window of 5 to infinity. Each sample, unless otherwise stated, was measured for 30 seconds at 4–7 minute intervals for a 40–60 minute period commencing immediately after the addition of zymosan. All incubations were performed in scintillation vials in the dark. Data are illustrated as the emission in counts per minute vs. time.

RESULTS

Effect of dapsone on CL. PMN were exposed to varying concentrations of dapsone for 30 minutes at 37°C in HBSS and then exposed to zymosan. CL was measured for 30 seconds at

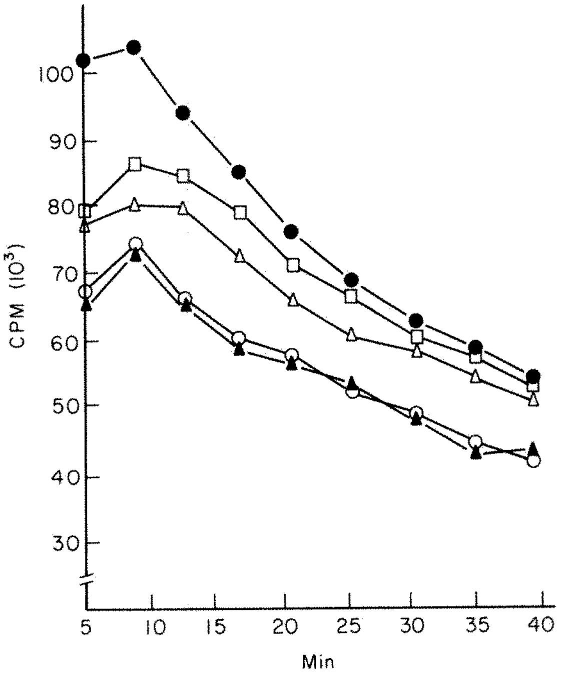

FIGURE 1. The effect of preincubation in dapsone on the CL response of human PMN. (●) PMN preincubated in ethanol-HBSS blank, (□) 0·5 μg/ml dapsone, (△) 1·0 μg/ml dapsone, (○) 5·0 μg/ml dapsone, (▲) 10 μg/ml dapsone.

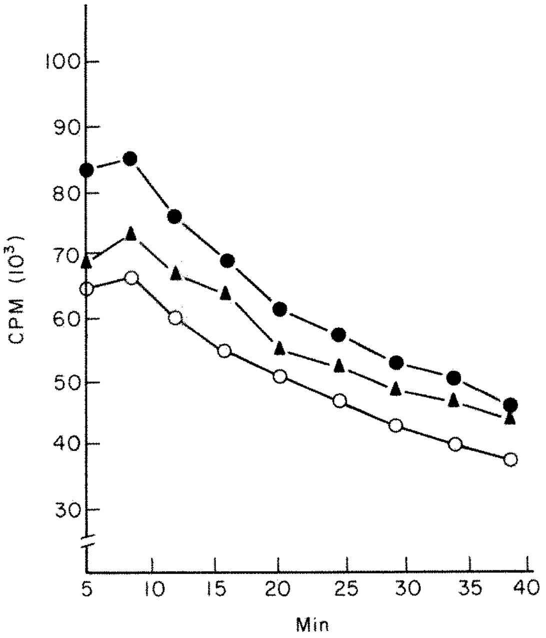

FIGURE 2. The effect of simultaneous addition of dapsone and zymosan. (●) ethanol-HBSS blank, (▲) 0·5 μg/ml dapsone, (○) 5·0 μg/ml dapsone.

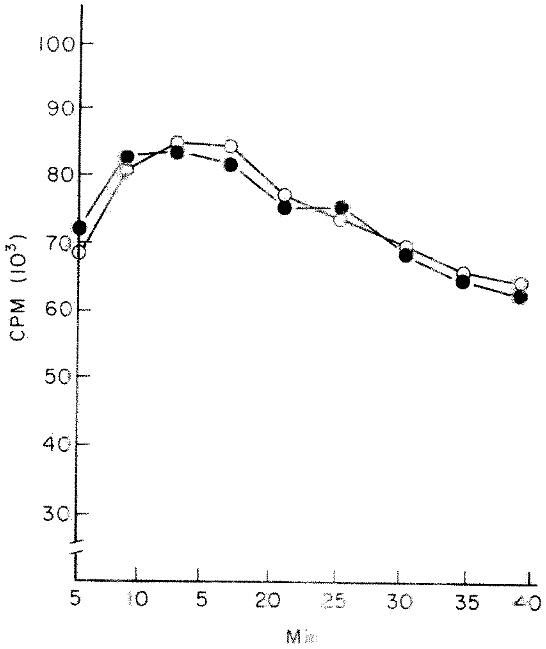

FIGURE 3. The effect of removal of dapsone after preincubation of PMN. (○) PMN preincubated with 5·0 µg/ml dapsone and then washed, (●) PMN preincubated in ethanol–HBSS blank and then washed.

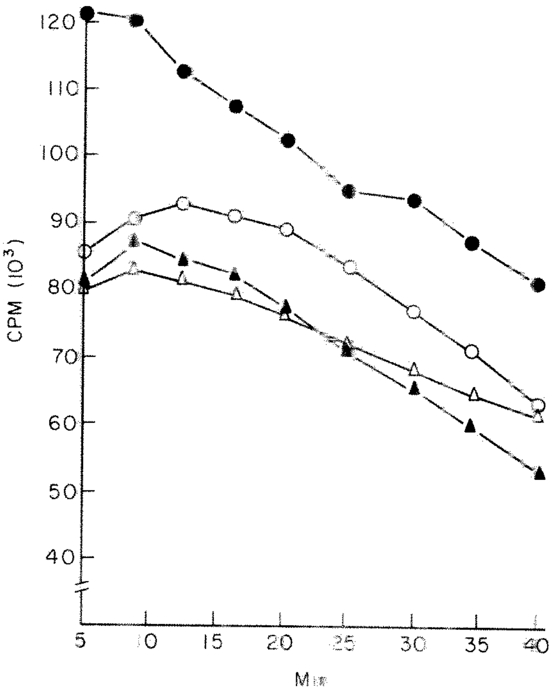

FIGURE 4. The effect of dapsone and/or azide on the CL response. (●) no dapsone or azide exposure, (△) 1·0 mM azide, (○) 5·0 µg/ml dapsone, (▲) 5·0 µg/ml dapsone and 1·0 mM azide.

6-minute intervals over a 40-minute period. PMN preincubated in HBSS alone or ethanol-HBSS served as controls. Dapsone at concentrations of 0·5 to 5·0 μg/ml inhibited PMN CL in a dose-dependent manner (Fig. 1). Higher doses (> 10 μg/ml) of dapsone did not inhibit CL greater than the 5 μg/ml dose. Simultaneous addition of dapsone and zymosan to PMN was as effective in reducing CL as preincubation with dapsone (Fig. 2). Preincubation of PMN with dapsone followed by washing away the drug resulted in the loss of dapsone-mediated CL inhibition (Fig. 3).

Sodium azide has been reported to decrease MPO activity and to decrease PMN CL. Azide (1 mM) depressed CL to the same degree as 5 μg/ml dapsone. Treatment of PMN with dapsone and azide together failed to produce a greater inhibition of CL than either agent alone (Fig. 4).

PMN viability. To establish that dapsone had no effect on PMN viability, Trypan Blue exclusion studies and morphological examination were performed on dapsone-treated PMN. After exposure to 10 μg/ml dapsone for 60 minutes at 37°C, PMN appeared morphologically normal and showed no increase in the number of cells taking up Trypan Blue.

Absorbance of 570 nm light. Anderson *et al.* (1977) reported that the CL spectrum of human PMN and MPO were nearly identical, with emission maxima at 570 nm. In order to rule out the possible quenching of CL by dapsone, the absorbance of up to 200 μg/ml dapsone in ethanol–HBSS at 570 nm was tested. Dapsone failed to produce detectable absorption at 570 nm.

DISCUSSION

Dapsone is particularly effective in the treatment of two inflammatory skin diseases of unknown aetiology, dermatitis herpetiformis and erythema elevatum diutinum (Katz *et al.*, 1976, 1977). Initiation of dapsone therapy prompts a rapid resolution of symptoms, and withdrawal of dapsone results in a rapid recurrence of disease. These kinetics suggest that the drug may be acting as a reversible inhibitor of one or more inflammatory systems. Since both of the aforementioned diseases display an early influx of PMN into the skin it is plausible that dapsone may exert a significant effect on PMN. In this study we have shown that therapeutically attainable concentrations of dapsone suppress PMN CL *in vitro*, and we have confirmed the inhibition of MPO by dapsone.

The inhibition of CL was dependent on the dosage of dapsone (below 5 μg/ml) and could be attributed neither to an artifactual quenching of emitted light nor to killing of PMN by the drug. Preincubation of PMN in dapsone followed by washing away the drug resulted in no inhibition of CL, indicating that dapsone must be present to effect CL inhibition and that the CL-generating mechanism is not permanently altered by dapsone treatment.

Several mechanisms have been implicated in the production of CL in human PMN. Cheung, Archibald & Robinson (1983) have found that inhibitors of prostaglandin synthesis may inhibit CL generation. Rosen and Klebanoff (1976) demonstrated that MPO-deficient PMN have a diminished ability to emit light. They also showed that addition of the MPO inhibitor azide produced a similar decrease of CL in normal PMN. Our finding that dapsone and azide produce an equivalent inhibition of CL that is not increased when both agents are simultaneously present, may be taken to indicate that both agents inhibit CL via the same mechanism, namely inhibition of MPO. That pretreatment with dapsone followed by removal of the drug was ineffective in inhibiting CL suggests that dapsone's action is reversible, in the manner of a competitive inhibitor. These findings are consistent with the data of Stendahl and colleagues (1978) who showed that dapsone competitively inhibits the halide component of the MPO system.

In recent years MPO has become appreciated as a potential modulator of inflammatory systems. The enzyme system has been shown to inactivate human alpha-1-proteinase inhibitor (Matheson *et al.*, 1981; Clark *et al.*, 1981), C5a, and the synthetic peptide chemotactic factor f-met-leu-phe (Clark & Szot, 1982). The beneficial effect of dapsone therapy in dermatitis herpetiformis may also be partially mediated through a facilitation of degradative functions (through increased proteolytic activity) allowing breakdown of the antigenic glutin/gliadin molecules to which the patients are believed to be sensitized (Lane, Huff & Weston, 1982), while simultaneously inhibiting the pro-inflammatory functions of neutrophils.

Harvath and co-workers (1983) recently reported that dapsone competitively inhibits the PMN-binding of the chemotactic peptide f-met-leu-phe, but not that of C5a or lymphocyte-derived chemotactic factor. As a connection between this activity of dapsone and the inhibition of MPO is not apparent, it may be that dapsone exerts its anti-inflammatory effects by more than one mechanism.

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Polyclonal B-cell activation and increased lymphocyte helper–suppressor ratios in discoid lupus erythematosus

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SUMMARY

We studied polyclonal B-cell activation in twenty-six patients with discoid lupus erythematosus (DLE). Spontaneous plaque-forming cells of the IgA class (IgA-SPFC) as determined by a reverse haemolytic plaque assay were significantly more common in patients with DLE than in fifty control subjects. The patients showed a positive correlation between IgA-SPFC and OKT4/8 ratios and also had a significantly higher mean OKT4/8 ratio. The two groups did not differ with regard to cells producing IgG or IgM or cells with OKT3, OKT4, OKT8 or OKM1 markers. None of the three patients with DLE who had IgA-SPFC values which were above the mean (+2 s.d.) for the control subjects had positive tests for ANA or low serum C3 or C4, but two of the three also had increased IgG-SPFC values. The results indicate that polyclonal B-cell activation occurs in a small proportion of patients with DLE.

Discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) are normally distinct entities but a clinical overlap is well recognized. Thus, discoid skin lesions were noted on presentation in 11% of 520 patients with SLE (Dubois, 1974) and occur at some stage of the disease in 14–20% (Estes & Christian, 1971; Dubois, 1974). Furthermore, patients with DLE may have mild general symptoms such as malaise and arthralgia and a proportion, estimated at between 2 and 20%, eventually develop SLE (Rowell, 1971; Bencze, 1970; Epstein & Tuffanelli, 1974).

Patients with SLE have polyclonal B-cell activation as shown by diffuse hyperglobulinaemia and autoantibodies of multiple specificities. The B-cell activation is also demonstrable *in vitro* in the form of increased immunoglobulin (Ig) production by unstimulated lymphocytes in culture (Jasin and Ziff, 1975; Nies & Louie, 1978) or increased spontaneous plaque-forming cells (Ginsburg, Finkelman & Lipsky, 1979), the latter being high particularly during active disease

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(Blaese, Grayson & Steinberg, 1980; Wangel, Milton & Egan, 1982). The B-cell activation may result from a decrease in the number of suppressor cells, defined by Fc gamma receptor or OKT8-positive lymphocytes or from reduced suppressor cell function (Bresnihan & Jasin, 1977; Krakauer *et al.*, 1980; Morimoto *et al.*, 1980), both of which have been reported in SLE. To our knowledge similar studies have not been done in patients with DLE. We report here the result of such a study which suggests that polyclonal B-cell activation occurs in a proportion of patients with DLE and correlates with an increase in the helper/suppressor cell ratio.

METHODS

Patients

There were twenty-six patients with DLE of whom four were male (Table 1). The lesions were clinically active in twenty-two and seven had arthralgia but none had other systemic symptoms. Five patients had positive ANA tests to a maximum titre of 1 in 100; none had anti-DNA antibodies. Skin biopsies had been done in twenty-four of the patients and showed Ig and/or complement deposition in the skin lesion in eighteen and in clinically unaffected skin in three patients. Two of these three had low titre ANA and reduced C4 but normal nDNA binding and no systemic symptoms apart from arthralgia.

The fifty control subjects were healthy blood donors.

Lymphocyte surface markers

The OKT3, OKT4 and OKT8 and OKM1 subsets were defined in sixteen patients and thirty control subjects by incubating Ficoll-Hypaque-isolated peripheral blood mononuclear cells (MNC) for 30 minutes on ice with the appropriate monoclonal antibodies (Ortho, Raritan, New Jersey) followed by two washes in ice-cold phosphate-buffered saline–bovine serum albumin (PBS–BSA) and a second 30-minute incubation with fluorescein isothiocyanate-conjugated rabbit anti-mouse globulin (Dakopatts, Copenhagen). After two further washes in PBS–BSA the MNC pellet was fixed in 3% paraformaldehyde and stored at 4°C in the dark until analysed by flow cytometry on a FACS IV.

Spontaneous plaque forming cells were determined in twenty-six patients and fifty control

TABLE 1. Clinical and laboratory features in twenty-six patients with DLE (IF = immunofluorescence studies)

Total	26
Male	4
Female	22
Active disease	22
Arthralgia	7
Other systemic symptoms	0
IF of lesion positive	18/24
IF of normal skin positive	3/24
ANA	5
Anti-nDNA	0
Reduced C3 or C4	4

subjects by a reverse haemolytic plaque assay (Wangel *et al.*, 1982) using staphylococcal protein A (Pharmacia, Uppsala, Sweden) coated sheep red blood cells (SPA–SRBC). Briefly, 25 μl of a 25% suspension of SPA–SRBC were mixed in microtitre wells with 100 μl of the MNC to be tested, 25 μl of anti-IgG, anti-IgA or anti-IgM (Dakopatts, Copenhagen) at concentrations of 1 in 25, 1 in 20 and 1 in 20 and 50 μl of SRBC-adsorbed guinea-pig complement diluted 1 in 6. The mixture was transferred from the wells to Cunningham chambers (Cunningham & Szenberg, 1968) which were sealed with paraffin, incubated at 37°C for 1 to 2 hours and for a further 1 to 2 hours at room temperature. Plaques were read under × 20 magnification and results expressed as SPFC per 10^6 MNC. Determinations were made in triplicate for each Ig class and negative controls lacking antiserum were included in each run. Throughout the study, red cells from the same two sheep were used for SPA coupling; the cells had in preliminary experiments been shown to give closely similar results. The same batch of SRBC-absorbed guinea-pig serum was also used throughout. The tests were done without knowledge of the clinical features of the patient.

Antinuclear antibodies were detected by indirect immunofluorescence on mouse liver. nDNA binding was measured by a Farr-type assay using the Medix-kit® (Medix, Kauniainen, Finland) and serum C3 and C4 levels were measured by a fluoronephelometric method (Larson, Gorman & Becker, 1973).

RESULTS

The patients with DLE had significantly higher numbers of spontaneous plaque-forming cells producing IgA (IgA-SPFC) than the control subjects (Table 2) but there was no difference between the two groups with regard to SPFC producing IgG or IgM. There was also a significant positive correlation between OKT4/OKT8 ratios and IgA-SPFC in the patients (Fig. 1), and the patients had a significantly higher OKT4/OKT8 ratio than the control subjects ($2{\cdot}04 \pm 1{\cdot}1$ vs $1{\cdot}4 \pm 0{\cdot}4$, $P < 0{\cdot}01$). IgA-SPFC and OKT4/OKT8 ratios did not correlate in the control subjects and there were no significant differences between patients and control subjects with respect to percentages of cells expressing OKT3, OKT4, OKT8 or OKM1 (data not shown).

Attempts to relate high IgA-SPFC numbers to indices of unusual activity or systemic disease failed to show a connection. Thus of the three patients with DLE who had IgA-SPFC values which were above the mean (+ 2 s.d.) for the control subjects, none had positive tests for ANA or low serum C3 or C4 and only one had arthralgia and positive immunofluorescence of clinically normal skin. Two of the three had IgG-SPFC values above the mean (+ 2 s.d.) for the control group but IgM-SPFC values were normal in all three.

TABLE 2. Spontaneous plaque-forming cells (10^6 MNC) of the three major Ig classes in control subjects and patients with DLE (mean ± s.d.)

	IgG-SPFC	IgA-SPFC	IgM-SPFC
Control ($n = 50$)	179·5 ± 208·7	517·9 ± 479·8	114·0 ± 117·2
DLE ($n = 26$)	143·5 ± 218·5	817·4 ± 555·5	93·0 ± 76·1
Student's t	0·17	2·36	0·43
P	NS	< 0·05	NS

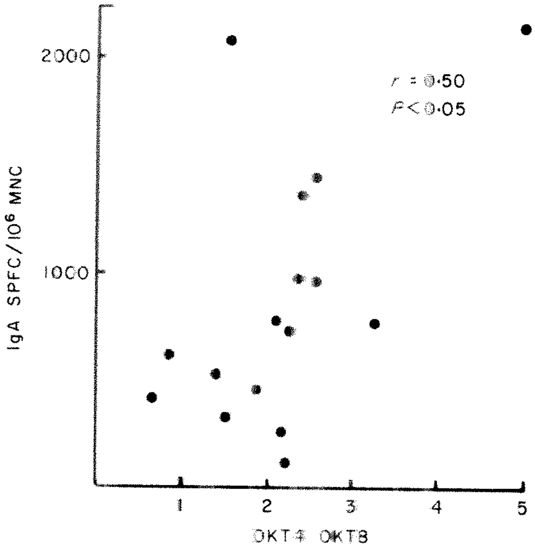

FIGURE 1. Correlation between OKT4/OKT8 ratios and IgA-SPFC in sixteen patients with DLE.

DISCUSSION

The main finding of the study was the raised number of IgA producing cells in the peripheral blood of patients with DLE compared with control subjects. Patients and control subjects did not differ in regard to SPFC of the other Ig classes but as we have previously shown, IgA-SPFC are usually the most numerous in normal subjects as well as in patients with inactive or with active SLE and discriminate best between the three groups (Wangel *et al.*, 1982).

Before accepting that a moderate degree of polyclonal B-cell activation occurs in DLE three alternative explanations for the association need to be considered. Firstly, the possibility that it is due to inadvertent and unrecognized inclusion of patients with SLE and discoid skin lesions into the study group is unlikely. The 19% prevalence of ANA in our group of patients is similar to or lower than the 13–50% reported by others (Weir, Holborow & Johnson, 1961; Shrank & Doniach, 1963; Beck & Rowell, 1966). Moreover, none of the three patients with the highest IgA-SPFC values had ANA, or low C3 or C4 and only one had Ig deposition in clinically normal skin. Secondly, intercurrent illnesses such as viral and gram-negative infections cause transient rises in IgA-SPFC (Wangel, unpublished observations); however, such infections were not obvious in our patients at the time of study. Thirdly, there was no correlation between the extent or activity of the skin lesions and IgA-SPFC values, making it unlikely that the high values merely reflected tissue injury.

If polyclonal activation is present at least in a proportion of patients with DLE the question arises whether it is a marker for a subset of patients who may later develop systemic disease. The present investigation provides no information on this point but a longitudinal study should settle the question.

The correlation between polyclonal B-cell activation and increased helper–suppressor cell ratios is not an unexpected finding in that either increased help or decreased suppression may result in B-cell activation. The lack of such a correlation in the control group may be partly because minor alterations in help and suppression are not accurately reflected by OKT4/OKT8 ratios and partly because minor changes in IgA-SPFC numbers in peripheral blood may not reflect the degree of polyclonal B-cell activation of the immune system as a whole.

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Effect of coal tar on cutaneous aryl hydrocarbon hydroxylase induction and anthralin irritancy

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SUMMARY

The inflammatory dose-response to topical anthralin and whole skin aryl hydrocarbon hydroxylase (AHH) activity were measured before and 24 h after application of a coal tar solution to the uninvolved skin of patients with psoriasis. The inflammatory response to anthralin decreased and total AHH activity increased after the tar treatment. A possible explanation is that anthralin or an irritant product is metabolized by AHH activity in the skin. Induction of AHH by coal tar increases its removal and reduces anthralin irritancy.

Aryl hydrocarbon hydroxylase (AHH) is part of the microsomal mono-oxygenase system required for the metabolism of a wide variety of drugs and foreign compounds by the liver, and is also detected in small amounts in other tissues. We have shown that AHH activity can be measured in full-thickness human skin biopsies (Finnen, Lawrence & Shuster, 1984) but not in epidermal suction blisters as originally claimed by Chapman *et al.* (1979), and it is induced two- to five-fold, *in vivo*, by topical pretreatment with coal tar solution (Bickers & Kappas, 1978; Finnen *et al.*, 1983).

The use of anthralin for chronic plaque psoriasis is often limited by irritancy. If this irritancy is due to an active metabolite of anthralin produced by a local enzyme system such as AHH, inflammation should be augmented by induction of enzyme activity by coal tar. Alternatively, if anthralin irritancy is due to the parent compound, an increase in cutaneous mono-oxygenase activity would speed local detoxification of anthralin and thereby reduce inflammation. We have therefore measured the effect of coal tar on anthralin irritancy and whole skin AHH activity.

METHODS

All the patients studied had chronic plaque psoriasis and agreed to take part after full explanation. Topical and systemic therapy was stopped for 2 weeks before the experiment. Clinically uninvolved skin and the same batch of anthralin were used throughout.

To investigate the effect of coal tar on anthralin irritancy coal tar solution BP was painted on to uninvolved volar forearm skin of ten patients (five female, mean age 41·4 years) and the methylated spirit vehicle on the opposite arm. These sites were left unwashed for 12 h and anthralin dissolved in chloroform was applied at 24 h (Lawrence, Howel & Shuster, 1984) using solutions freshly made before each experiment. Serial dilutions of anthralin ranging from 160 μg to 2·5 μg and a chloroform control were applied in 10 μl volumes to aluminium patch test pads and the chloroform allowed to evaporate off. These patches were then applied to clinically normal forearm skin and occluded with microporetape for 24 h. The corresponding site for each concentration was used on the control side. The irritant response to anthralin was measured by the change in skin fold thickness with Harpenden calipers (Lawrence & Shuster, in preparation) immediately before and 24 h after removal of the patch test pads, at which time inflammation was maximal.

To investigate the possible role of AHH activity in this response we measured whole skin AHH activity and anthralin irritancy with and without coal tar application in another group of patients. In this part of the study we used anthralin in emulsifying ointment with 0·5% salicylic acid as a stabilizer, since it avoided the need for making fresh solutions before each experiment. Although this base is not pro-inflammatory (Kersey *et al.*, 1981), irritancy is enhanced in an ointment base (Lawrence *et al.*, 1984).

Twelve patients (10 female, mean age 32 years) were studied. Coal tar BP was applied as before to the clinically normal forearm skin. Anthralin (BP) 0·8%, 0·1% and 0·025% and 0% (base control) in emulsifying ointment and 0·5% salicylic acid was spread on aluminium patch test pads which were then placed on the forearm skin in random order and occluded and measured as before; two patches were used for each concentration and the results were averaged.

Basal and induced whole skin AHH activity was measured in six of these patients. Coal tar solution BP was painted on to an area 10 cm in diameter on the clinically normal anterior abdominal wall skin. Eighteen to twenty four hours later a skin ellipse, approximately 1 × 3 cm, was outlined in ink and a transparent adhesive tape impression of the area to be excised taken. The surface area was then measured using a planimeter. A full-thickness excision of the whole of the marked area was made. A similar biopsy was taken from the opposite side of the abdomen which had been pretreated only with the methylated spirit vehicle. The mean sample size was 387 ± 49 mg (s.e.) and 2·74 ± 0·14 cm^2. AHH activity was measured in whole skin homogenates as described previously (Finnen *et al.*, 1983).

Anthralin irritancy results were assessed by analysis of variance of the dose-response curve excluding the control and by Student's paired *t*-test for each concentration used.

RESULTS

There was no detectable irritant effect of applying coal tar solution or methylated spirit to the forearm skin of any patient studied. The effect of coal tar application on anthralin irritancy, using anthralin in chloroform, as shown in Fig. 1. Skin irritancy, measured by the change in skin fold thickness, was less from 5–160 μg anthralin, and this difference was significant ($P<0{\cdot}05$) at 20, 80 and 5 μg ($P<0{\cdot}01$). Analysis of variance of the linear regression of the full dose-response curve showed a significant ($P<0{\cdot}05$) decrease in anthralin irritancy in skin pretreated with coal tar solution compared with untreated skin.

Using anthralin in emulsifying ointment and 0·5% salicylic acid (Fig. 2), anthralin irritancy was greater than with similar concentrations of anthralin dissolved in chloroform (Fig. 1) as found previously (Lawrence *et al.*, 1984). After coal tar application anthralin irritancy was reduced at each concentration used. This difference was significant ($P<0{\cdot}05$, Student's *t*-test)

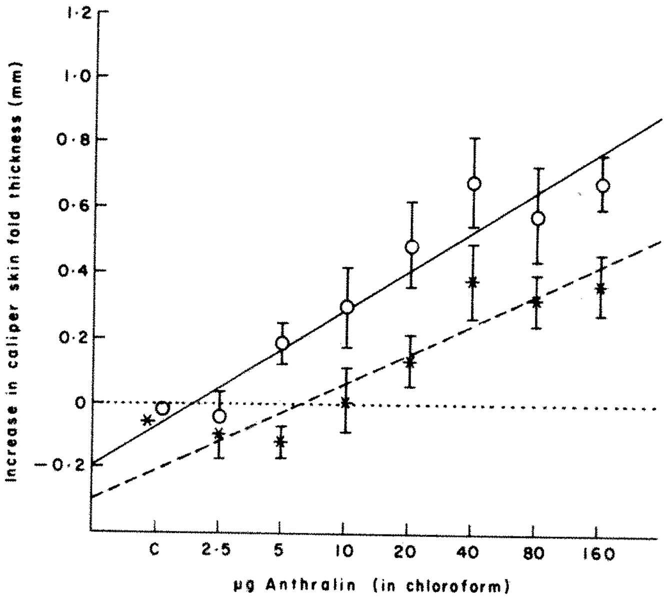

FIGURE 1. Anthralin irritancy measured by the increase in caliper skin fold thickness, in skin pretreated with coal tar solution (*) and untreated (O). Differences were significant ($P < 0{\cdot}05$, paired Student's *t*-test) at 20 and 80 μg, and ($P < 0{\cdot}01$) at 5 μg of anthralin in chloroform. Bars give mean ± s.e.m. Regression lines calculated from $y = a + b \log_2 x$: untreated ——; coal tar treated – – –.

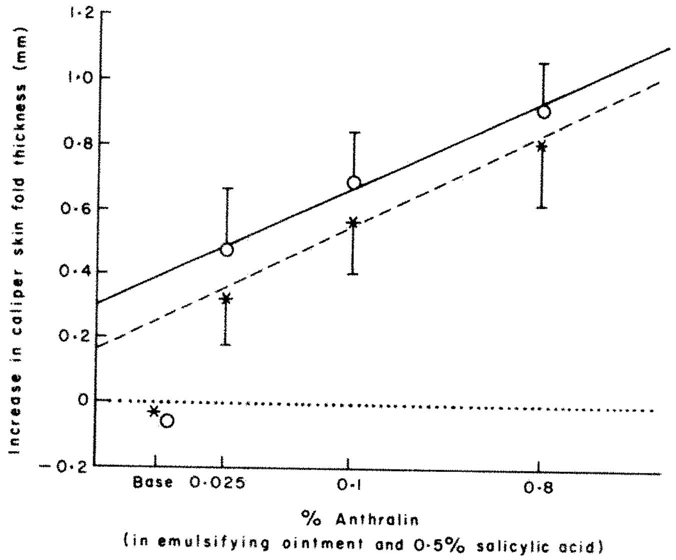

FIGURE 2. Anthralin irritancy using anthralin in emulsifying ointment and 0·5% salicylic acid, measured by the increase in caliper skin fold thickness, in skin pretreated with coal tar solution (*) and untreated (O). Differences were significant ($P < 0{\cdot}05$) at 0·25%. Bars give mean ± s.e.m. Regression lines calculated from $y = a + b \log_2 x$: untreated ——; coal tar treated – – –.

at 0·025% but comparison of the full dose-response showed no significant difference ($P > 0{\cdot}05$ analysis of variance).

In clinically normal abdominal wall skin, basal AHH activity was only detected in four of the six subjects but after tar application AHH activity was detected in all six. The increase was three- to sixfold, from a mean of 0·14 ± 0·03 (mean ± s.e.) pmol/min/g tissue to 0·75 ± 0·23 pmol/min/g tissue after coal tar induction (Table 1).

TABLE 1. Whole skin AHH activity (pmol/min/g tissue) in six patients 18–24 h after application of coal tar solution BP and methylated spirit base

Patient No.	18–24 h after methylated spirit application	18–24 h after coal tar solution BP application
2	0·13	0·75
3	0·19	0·68
8	not detected	1·8
10	0·18	0·77
11	0·07	0·39
12	not detected	0·12

DISCUSSION

We have shown that the application of coal tar solution to clinically normal psoriatic skin results in a significant reduction of irritancy from anthralin applied in chloroform. In the ointment base, irritancy was reduced after coal tar application at each concentration used, although the difference was only significant at the lowest concentration. In emulsifying ointment and 0·5% salicylic acid, anthralin irritancy was greater than in chloroform for comparable concentrations of anthralin. This is in part due to the greater quantities of anthralin applied but also presumably due to enhanced absorption since the maximal anthralin irritancy response is greater with an ointment base than with a chloroform base (Lawrence *et al.*, 1984). At the same time as anthralin irritancy decreased after coal tar application, there was an increase in whole skin AHH activity of the same order as that previously described by us (Finnen *et al.*, 1984) and others (Bickers & Kappas, 1978). If, as seems likely, mono-oxygenases such as AHH metabolize anthralin in the skin, the present findings would suggest that anthralin irritancy is not due to a metabolite but to the toxic parent compound which is inactivated faster in skin with induced AHH activity. If, by contrast, the therapeutic activity of anthralin required prior metabolism then induction of cutaneous mono-oxygenases would greatly increase the therapeutic ratio of anthralin and we are now exploring this approach.

ACKNOWLEDGMENT

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Clinical features of superficial fungal infections caused by *Hendersonula toruloidea* and *Scytalidium hyalinum*

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SUMMARY

The clinical appearances of infections in 128 patients caused by the mould fungi *Hendersonula toruloidea* (102) and *Scytalidium hyalinum* (37) are described. All the patients originated from the tropics or subtropics but those infected with *S. hyalinum* came from either the Caribbean or West Africa. Forty-one per cent of those studied had a concurrent dermatophyte infection. While the clinical features of *Hendersonula* and *Scytalidium* infections resembled those seen in 'dry type' *Trichophyton rubrum* infections, there were a number of distinctive features. These included the absence of dorsal infection on the feet, lateral and distal onychomycosis with extensive onycholysis and the development of paronychia on the fingers. The majority of affected patients showed no serious underlying abnormality or predisposing skin disease which could facilitate invasion. The routine incorporation of cycloheximide, which inhibits the growth of these organisms, in mycological media may explain why they are infrequently diagnosed.

In 1970 Gentles and Evans described in eight patients a form of dermatomycosis and onychomycosis which was clinically indistinguishable from a dermatophyte infection. The causative organism was a darkly pigmented mould, *Hendersonula toruloidea*, which was inhibited by the cycloheximide normally incorporated in mycological culture media and could be isolated only in the absence of this antibiotic. Subsequently there have been reports of this infection in patients originating from the tropics or subtropics living in the United Kingdom (Kurwa & Campbell, 1972; Campbell *et al.*, 1973; Eady & Moore, 1974; Moore, 1978; Turvey, English & Phillips, 1979), Denmark (Rosman, 1977), India (Singh & Barde, 1980), France (Badillet, de Bievre & Spizajzen, 1982), and Canada (Carruthers, Stein & Black, 1982). These superficial infections have always involved thickly keratinized sites such as the skin of the palms and soles or the nails. More recently a similar infection has been described, caused by a white or cream-coloured mould, *Scytalidium hyalinum*, which is also inhibited by cycloheximide (Campbell & Mulder, 1977; Moore, 1978; Peiris, Moore & Marten, 1979).

Hendersonula toruloidea is a recognized plant pathogen, which has been isolated from a variety of hosts in the southern and eastern United States, the Caribbean, Africa, the Near East and Asia, but is not indigenous to the United Kingdom (Punithalingam & Waterston, 1970). *Scytalidium hyalinum*, in contrast, has never been isolated from the environment. Since 1972 a considerable number of patients with infections caused by these organisms have been seen at St John's Hospital for Diseases of the Skin. The clinical and epidemiological data from 128 patients are reviewed.

METHODS

All of the patients were seen at the out-patient department of St John's Hospital for Diseases of the Skin although twelve individuals had been referred from other hospitals after the diagnosis had been made. Skin and nail samples were taken from clinically affected sites and were examined by microscopy in 30% potassium hydroxide. Material was cultured on Sabouraud's dextrose agar (SDA: dextrose 40 g l^{-1}; Oxoid mycological peptone 10 g l^{-1}; agar 15 g l^{-1}) supplemented with chloramphenicol (0·05 g l^{-1}) for the isolation of *H. toruloidea* (HT) and *S. hyalinum* (SH) and on SDA supplemented with chloramphenicol (0·05 g l^{-1}) and cycloheximide (Actidione 0·5 g l^{-1}) for the isolation of dermatophytes. Incubation was at 28°C for 14–21 days.

The characteristic appearance of the non-dermatophyte hyphae in skin and the morphological features of the fungi in culture have been described elsewhere (Campbell *et al.*, 1973; Campbell & Mulder, 1977; Moore, 1978).

RESULTS

Organisms isolated

For purposes of assessment an infection was judged to be present only if direct microscopy and culture were both positive for each site assessed. The organisms and combinations of organisms isolated are shown in Table 1.

Sixty-two patients (48%) yielded more than one pathogen on culture at the same or at different sites, ten patients (7·8%) yielding both non-dermatophytes and 52 (41%) patients yielding a dermatophyte in addition to one or both non-dermatophytes.

TABLE 1. Organisms isolated from 128 patients with non-dermatophyte infections

Organisms isolated	Number of patients
H. toruloidea alone	52
S. hyalinum alone	14
H. toruloidea and *S. hyalinum*	10
H. toruloidea and dermatophyte*	39
S. hyalinum and dermatophyte*	12
H. toruloidea and *S. hyalinum* and dermatophyte*	1
Total	128

*Dermatophyte species isolated: *Trichophyton rubrum* (twenty-eight patients); *Trichophyton interdigitale* (seventeen patients); *Epidermophyton floccosum* (six patients); *Trichophyton soudanense* (one patient).

TABLE 2. Geographic distribution of *H. toruloidea* and *S. hyalinum* isolations*

	H. toruloidea	*S. hyalinum*
Caribbean	40	35 (46%)
West Africa	7	3 (33%)
East Africa	6	—
Indian Subcontinent	40	—
Far East and West Pacific	5	—
Mauritius	4	—

* Eleven patients had mixed HT and SH infections.

Patient data

There were 107 males and 21 females whose ages ranged from 19 to 72 years. All had been born outside the United Kingdom and had presented between 1 and 30 years (mean 14 years) after arriving in Great Britain. Eighty-two (64%) of those interviewed had visited their country of origin for short periods subsequently.

The countries of origin of the patients infected by the different organisms are presented in Table 2. It was noted that all the patients who yielded *S. hyalinum* came from the Caribbean or West Africa.

Clinical features

Eighty-four of the patients (66%) presented with other skin lesions and the fungal infection was a secondary finding. The other skin diseases diagnosed included eczema (27), pruritus (12), palmoplantar hyperkeratosis (8), lichen simplex (7), urticaria (3), psoriasis (2), lichen planus (2) and leprosy (2). Only two patients, however, had serious underlying systemic disease: chronic active hepatitis in one case and systemic lupus erythematosus in the other. The clinical features of the infections in these patients were unremarkable.

The remaining forty-four patients (34%) presented with signs or symptoms directly attributable to the fungal infection, the commonest of them being nail dystrophy. Itching was recorded at the site of infection in seventy patients (55%).

Toe web infections were diagnosed in 100 patients (HT – 72; SH – 24; HT + D – 3; SH + D – 1). As the same site was not necessarily examined in every patient the true prevalence of infection in each site could not be determined. Regardless of whether a pure or mixed infection was present the clinical appearances were similar to dermatophytosis, with scaling or erosions of the interdigital skin being the main signs. Itching was inconstant and maceration only occurred in four patients. Blister formation was not recorded.

Infections of the sole were demonstrated in seventy-seven cases (HT – 61; SH – 12; H + D – 4; SH + D – 0) and were very similar to the dry or moccasin type of *T. rubrum* infection (Fig. 1), although secondary involvement of the dorsum of the foot did not occur. The inflammation was variable but some patients who were infected with a non-dermatophyte alone showed a clear margin of erythema along the lateral or medial border of the foot.

Palmar infection was diagnosed in eighteen patients (HT – 9; SH – 9) and in nine patients it was unilateral. Fine scales were distributed across the palm but were most prominent in the

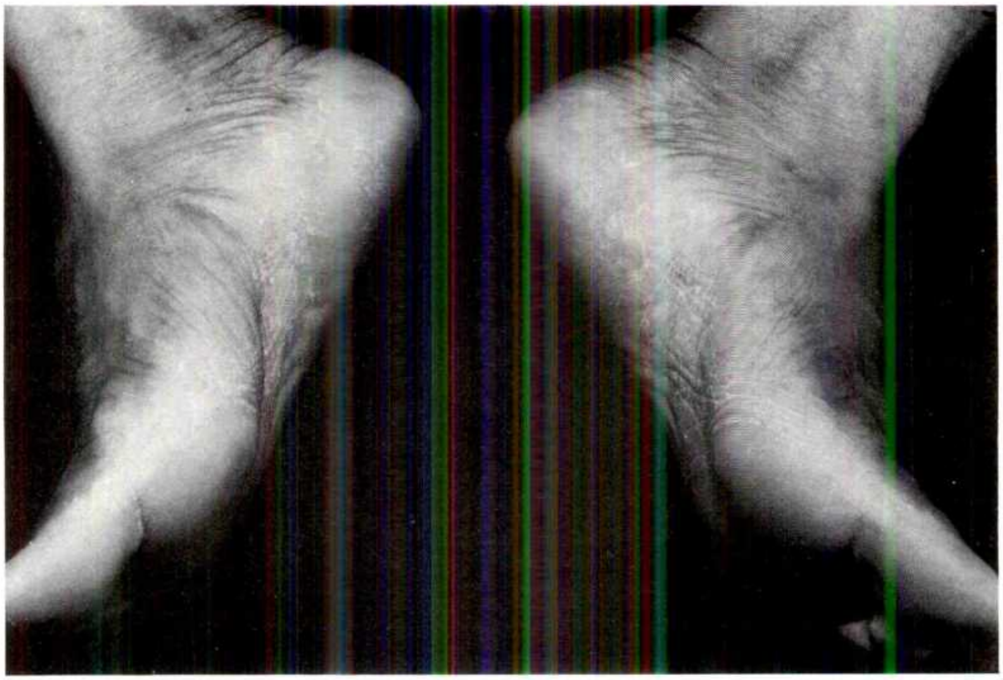

FIGURE 1. *S. hyalinum* infection on the soles.

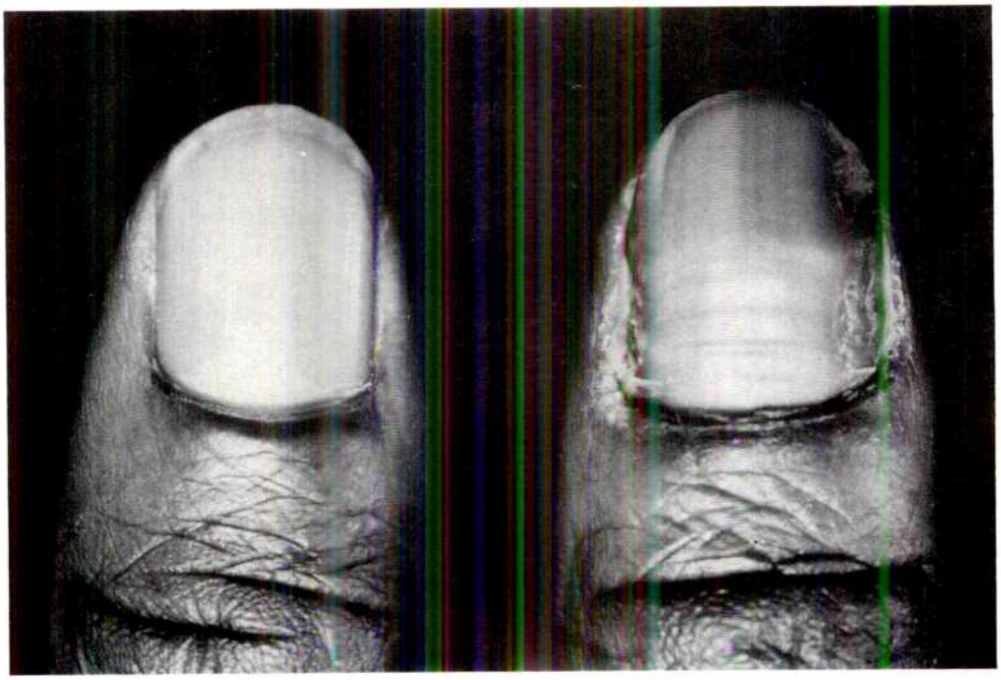

FIGURE 2. Early onychomycosis due to *H. toruloidea*: lateral nail plate invasion (thumb).

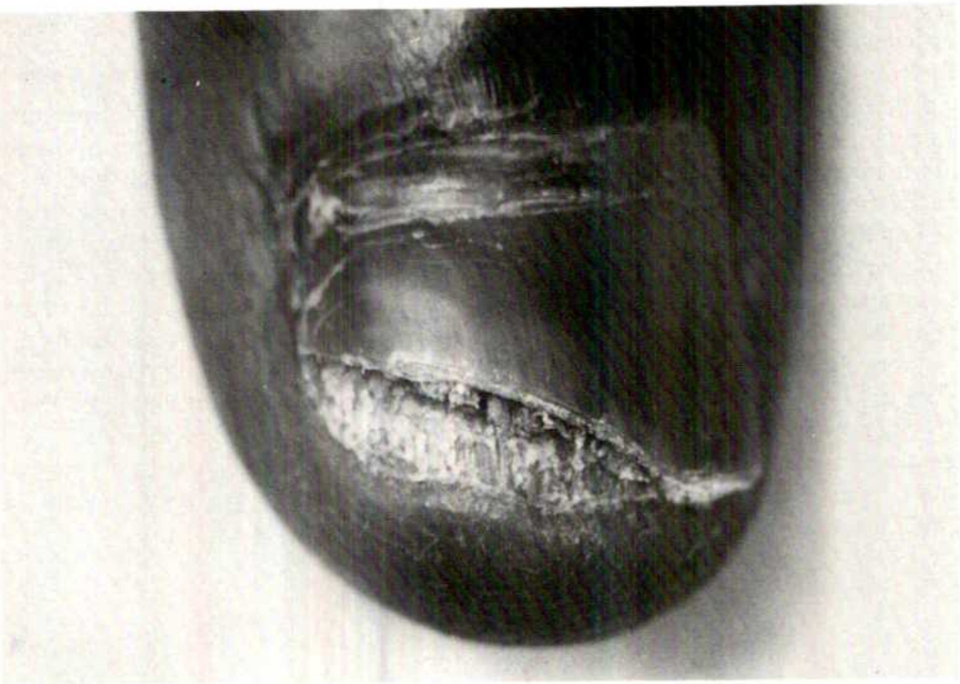

FIGURE 3. Onychomycosis due to *H. toruloidea*: later stage with hyperkeratosis and onycholysis (thumb).

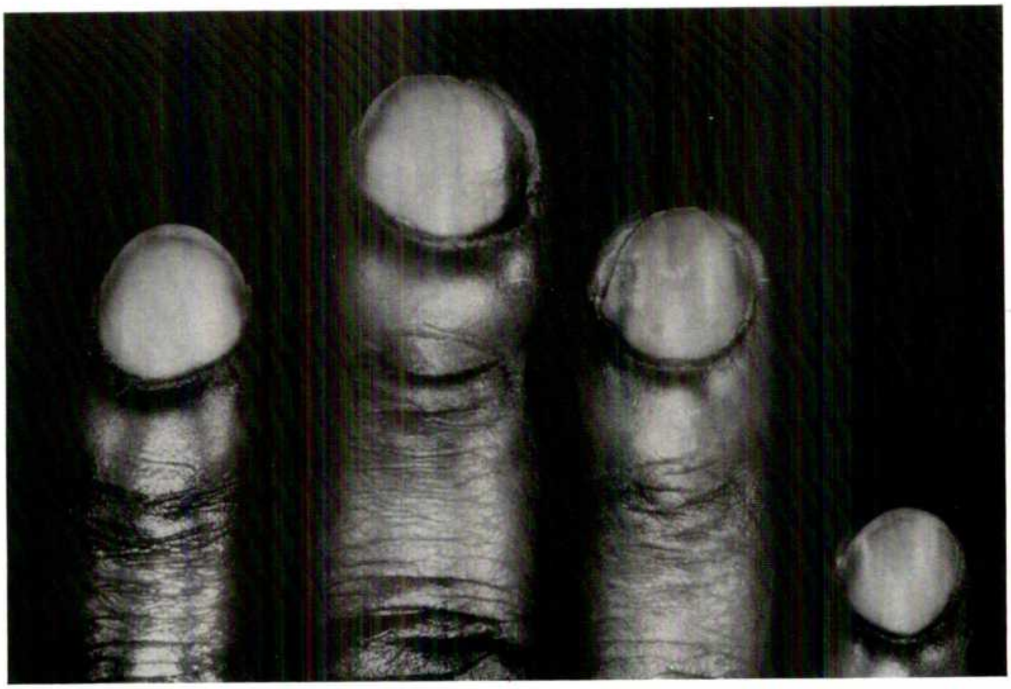

FIGURE 4. Paronychia associated with *H. toruloidea* infection. Note lateral nail plate invasion (fingers).

palmar creases. In all cases there was coexistent infection of the sole. Vesicles were seen in neither palms nor soles.

Both finger-nails (ten patients: HT – 9; SH – 1) and toe-nails (fifty-seven patients: HT – 45; SH – 12) were affected. The main pattern of nail plate invasion was distal and lateral subungual onychomycosis (Zaias, 1972). None of the patients developed superficial white onychomycosis. As in dermatophytosis thickening and opacification of the nail plate and onycholysis were seen. Several features, however, most commonly displayed in finger-nails appeared particularly associated with non-dermatophyte infection. Onycholysis of the lateral nail plate was often the predominant form of nail invasion (Fig. 2) and could spread to involve the whole nail plate (Fig. 3) without significant nail plate thickening, leading to a complete fracture and shedding of the major portion of the nail. Additionally, although Candida species were cultured from only three cases, paronychia were often present (Fig. 4) and in some instances hyphae typical of *H. toruloidea* or *S. hyalinum* were seen in nail fold debris. Candida was not isolated from these patients suggesting that the non-dermatophytes themselves can invade nail folds.

These features of onychomycosis due to *Hendersonula* and *Scytalidium* are more suggestive of candidosis than dermatophytosis. In toe-nail infections the appearances cannot be distinguished from those caused by dermatophytes except in early cases.

DISCUSSION

Infections caused by *H. toruloidea* and *S. hyalinum* are not uncommonly diagnosed at St John's Hospital. The fact that symptoms, apart from nail dystrophy, are often minimal, may explain why so little attention has previously been given to these infections.

Although one of the original patients described by Gentles and Evans (1970) was a native Scot, all the affected individuals in the present survey originated from the tropics or subtropics, suggesting that the infections are usually imported. Unfortunately, no surveys have been carried out to determine the prevalence of these infections in potential endemic areas. Failure to isolate the organisms may also be related to the inclusion of cycloheximide in the culture media, used in the majority of investigations of superficial mycoses in the tropics. Additionally, no surveys of normal populations in the United Kingdom have been published which might determine the prevalence of these fungi in the community.

The factors which lead to the invasion of the stratum corneum or nails by *H. toruloidea* or *S. hyalinum* are unknown. Subcutaneous infection by *H. toruloidea* has been reported only twice, the fungus having been isolated from a deep facial lesion in a patient with a known cell-mediated immune deficiency (Mariat *et al.*, 1978) and from a mycetoma affecting the foot (Drouhet & Dupont, 1983). However, the majority of patients show no serious underlying abnormality, such as immunosuppression or peripheral vascular disease or even predisposing skin conditions of the infected sites which would facilitate invasion. The organisms appear to be capable of invading healthy skin and nails, although they are normally confined to thickened keratin, suggesting that in other sites host defence mechanisms can easily overwhelm the organisms.

No clinical differences could be seen between infections caused by *H. toruloidea* and *S. hyalinum*. The clinical features described are similar to those common seen in *T. rubrum* infections of peripheral sites but some diagnostic points such as the prominent lateral nail plate invasion with onycholysis and paronychia are summarized in Table 3.

Both *H. toruloidea* and *S. hyalinum* appear well adapted to cause disease in man, and in the light of the lack of response of these infections to topical therapy, griseofulvin and ketoconazole

TABLE 3. Onychomycosis due to *Hendersonula* and *Scytalidium*

Changes similar to dermatophytosis	Changes uncommon in dermatophytosis
Nail plate thickening	Lateral nail plate invasion alone
Opacification	Paronychia
Onycholysis	Transverse fracture of the proximal nail plate
Involvement of palms, soles or toe-webs	

(Hay, 1983) it remains to be seen whether they will become endemic in temperate countries such as the U.K. in a similar fashion to anthropophilic dermatophytoses causing tinea pedis (Gentle & Holmes, 1957; English & Gibson, 1959).

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The culture of dermal papilla cells from human hair follicles

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SUMMARY

Dermal papillae were isolated from human hair follicles and primary cell cultures were established from the papilla explants. The cultured papilla cells spread slowly, initially as a monolayer, and eventually formed multi-layered parallel arrays of fibroblast-like cells. At the edges of expanding colonies the cells were large and flattened and showed a tendency to form clumps. The behaviour of human dermal papilla cells in culture is very similar to that reported in cultures of papilla cells from rat vibrissa follicles.

The study of human hair growth and hair disease has been hampered by the difficulty of obtaining sufficient tissue from the intact follicle on which to perform various research techniques. In 1981 Weterings *et al.* described a method for culturing epithelial cells derived from the outer root sheath of the human hair follicle, and subsequently Wells (1982) reported culturing epithelial cells from human telogen hair bulbs. Jahoda and Oliver (1981) have reported the establishment of *in vitro* cultures of dermal papilla cells from rat vibrissa follicles. In this study a method for isolating intact dermal papillae from human hair follicles is described. Primary cell cultures were then established from the papilla explants.

METHODS

Isolation of dermal papillae

Normal skin was obtained with the surgical excision of various benign tumours from the scalp. The excised tissue was placed in culture medium in a sterile Petri dish. All microsurgical procedures were performed under a binocular dissecting microscope. Each anagen follicle was dissected free from the surrounding tissue and transected about 1 mm above its base. The hair bulb was then transferred to a second medium-containing Petri dish. For the following stages a 21G needle attached to a 2 ml syringe was used. A second needle or a pair of watchmaker's forceps was used for holding the follicle. The epithelium was separated from the fibrous sheath and the dermal papilla by the application of gentle pressure to the proximal end of the follicle with the rounded tip of the needle. Pretreatment with trypsin was not necessary. The fibrous sheath was then incised proximally using the cutting edge of the needle bevel, and by further gentle manipulation the dermal papilla could be persuaded to emerge through the slit. The papilla was transected across its stalk and transferred on the needle tip to the culture vessel.

Electron microscopy

In order to confirm that the isolated dermal papillae were free from epithelial contamination, several papillae were fixed in 3% glutaraldehyde and processed for electron microscopy.

Cell culture

All materials for cell culture were purchased from Flow Laboratories. Papilla explants were placed in 35 mm plastic Petri dishes, generally four or five in each dish, and allowed to float freely in the culture medium. In some experiments papillae were either anchored under glass coverslips or placed in only a small volume of culture medium. The culture medium was 199 containing penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mmol/ml) and supplemented with 20% fetal bovine serum. The medium was changed every third day after the culture was established. Cultures were established and maintained in a humidified atmosphere containing 5% CO_2.

RESULTS

An isolated papilla is shown in Fig. 1. No epithelial contamination was seen using phase-contrast light microscopy and this was confirmed at electron microscopy. The plane of

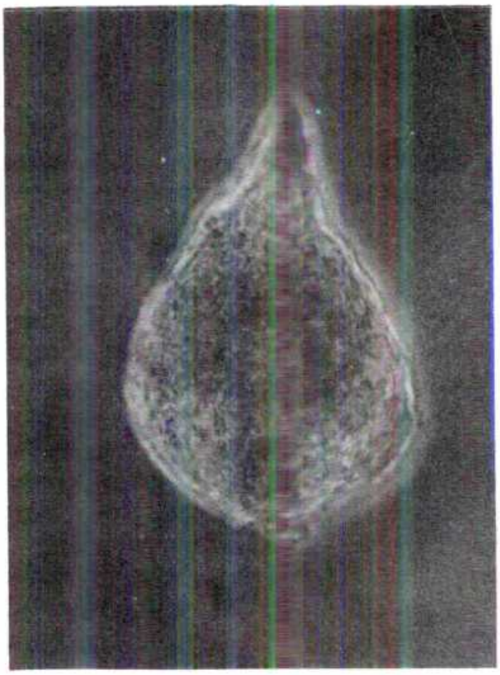

FIGURE 1. An isolated dermal papilla. (Phase contrast, × 145.)

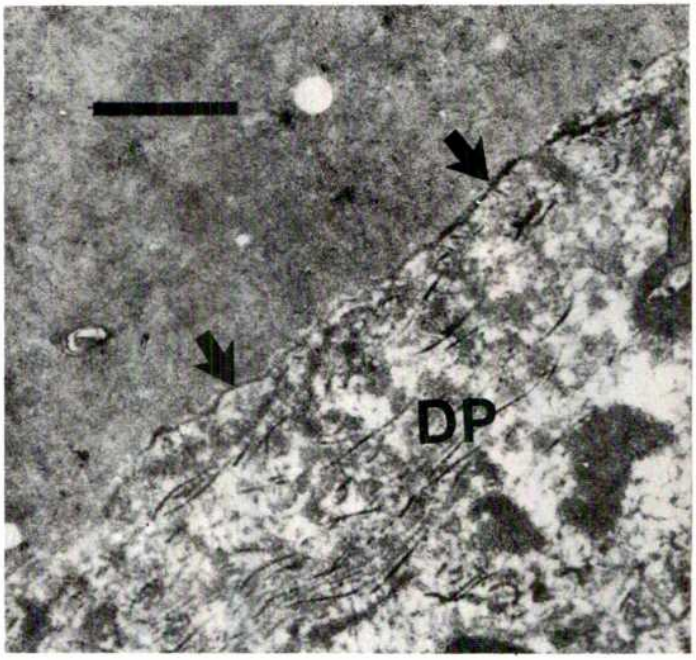

FIGURE 2. Electron micrograph of an isolated dermal papilla (DP) showing absence of epithelial cell contamination. The lamina densa (arrows) remains attached to the papilla. Bar = 1 μm.

cleavage between the dermal papilla and the follicular epithelium was within the lamina lucida of the basement membrane. The lamina densa remained attached to the dermal papilla (Fig. 2). After 5–6 days the papilla explants became rounded and attached to the plastic substrate. Successful attachment occurred only where explants were allowed to float freely in the medium. Papillae anchored under coverslips or placed in small volumes of medium did not attach. Within 24 hours of attachment cells began to migrate from the explant on to the plastic substrate. Following cell migration there was a delay of 1–3 weeks before proliferative activity became apparent. To date six proliferating cell cultures have been established from human dermal papillae. In an equal number the migrated cells died before proliferation took place. Cells spread slowly across the substrate, initially as a monolayer. At the periphery of these enlarging colonies the papilla cells were large and flattened, and often exhibited numerous cytoplasmic processes. The cytoplasm often had a striated appearance. Some of these cells were multinucleated. As the population density increased the cultures assumed a more typical fibroblast morphology and formed into parallel arrays of spindle-shaped cells which eventually became multilayered and then peeled away from the substrate. After 3–4 weeks in culture clumps of cells appeared around the edges of the outgrowth, usually where the cell density was quite low. These clumps resembled attached papilla explants (Fig. 3). Papilla cell cultures have been maintained for up to 12 weeks. However, none has progressed to confluence because of the tendency of papilla cells to form multilayered aggregates rather than spreading as a monolayer. This could probably be overcome by using a larger number of papillae in each dish. A limited number of subcultures have been performed using fragments lifted off the primary culture. Cells in the first passage appeared to retain the morphology and growth characteristics that were seen in primary culture.

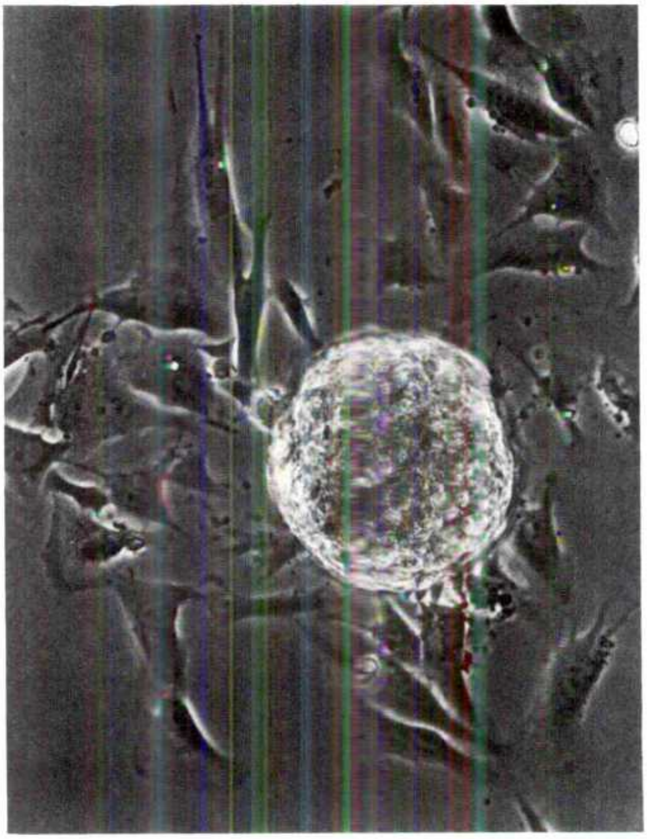

FIGURE 3. Dermal papilla cell culture at 4 weeks showing clumping of cells at periphery of outgrowth. (Phase contrast × 230.)

DISCUSSION

The dermal papilla is a highly specialized structure which remains as a discrete unit throughout successive hair cycles. Dermal papilla cells normally show very low proliferative activity (Pierard & De La Brassinne, 1975). *In vivo* studies have shown that the dermal papilla is necessary for the induction and maintenance of the follicular development (Cohen, 1961; Oliver, 1967, 1970) and it is probable that the dermal papilla also mediates control of the hair cycle. The slow growth and tendency to form papilla-like clumps suggest that papilla cells retain at least some of their *in vivo* properties in cell culture. The behaviour and morphology of human papilla cells in culture are similar to what has been described with cultured papilla cells from rat vibrissa follicles (Jahoda & Oliver, 1981), reinforcing the idea that the dermal papilla contains a functionally unique population of fibroblast-like cells. The ability to grow human papilla cells *in vitro* will allow greater scope for the study of their role in relation to hair growth. Cultured papilla cells may also prove valuable for studying hair disease in which papillary dysfunction may be involved.

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Pharmacology and Treatment

Treatment of chronic actinic dermatitis with azathioprine

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SUMMARY

Fourteen patients with severe unremitting chronic actinic dermatitis were treated with oral azathioprine in a dosage of 100–200 mg daily for a mean 11·5 months. Clinical improvement, apparently permanent, occurred after a few weeks to several months and continued for up to 2 years. Nine patients cleared or improved markedly, one patient cleared and then relapsed again on treatment, two patients showed no response and two patients needed to discontinue therapy because of gastrointestinal side-effects. We feel that in many patients azathioprine can be a very effective therapy for this extremely incapacitating disease.

Chronic actinic dermatitis (CAD) (Hawk & Magnus, 1979) is a severe, socially disabling and intractable photosensitive dermatitis particularly affecting elderly men, and has been shown to be a spectrum of diseases including actinic reticuloid (Ive *et al.*, 1969), photosensitive eczema (Ramsay & Kobza Black, 1973) and the photosensitivity dermatitis/actinic reticuloid (PD/AR) syndrome (Frain-Bell *et al.*, 1974). It is characterized by a light-sensitive predominantly eczematous eruption sometimes with infiltrated plaques on the exposed areas and a tendency to progress to generalized erythroderma, sometimes with circulating Sézary cells in severe cases (Johnson, Cripps & Norback, 1979; Neild *et al.*, 1982; Zugermann, Beeaff & Roenigk, 1980), though this latter condition seems to be benign (Chu *et al.*, 1983; Neild *et al.*, 1982). The histological appearance is usually eczematous, although sections from plaque areas may show a more marked reaction occasionally simulating a cutaneous lymphoma (Ive *et al.*, 1969; Neild *et al.*, 1982). Abnormal cutaneous reactions to ultraviolet and occasionally visible radiation are also present, the most frequent abnormalities being lower than normal minimal erythema doses (MEDs) and exaggerated papular responses (Frain-Bell *et al.*, 1974; Hawk & Magnus, 1979; Ive *et al.*, 1969; Ramsay & Kobza Black, 1973). CAD is usually very persistent, and it seems probable that very occasionally it may transform to a cutaneous lymphoma (Jensen & Sneddon, 1970; Perrot *et al.*, 1978; Thomsen, 1977) whereas other cases can resolve spontaneously (Hawk & Magnus, 1978). Its cause is unknown, although it has been suggested that contact sensitivity may play a part (Frain-Bell, Hetherington & Johnson, 1979), and some cases at least can probably be attributed to chemical or drug photosensitivity (Zugermann, 1981; Wojnarowska, Calnan & Hawk, 1982), or even to so-called persistent light reaction (Wilkinson, 1962; Jillson & Baughman, 1963).

Treatment of CAD has been very difficult but there have been suggestions of response to azathioprine (August, 1982; Roed-Petersen & Thomsen, 1980). We have therefore retrospectively reviewed a series of fourteen patients with CAD who have been treated with that drug.

METHODS

Patients (Table 1)

Fourteen consecutive severely affected patients who had the characteristic clinical, histological and photobiological abnormalities of chronic actinic dermatitis and in whom no aetiological sensitizing agents were found during patch and photopatch testing were treated with azathioprine in a dose of 100–200 mg daily in the out-patient photobiology clinic at St John's Hospital for Diseases of the Skin, London. They had not responded to at least some months of simple treatment measures such as restriction of light exposure, use of sunbarrier preparations and topical steroid therapy. The ages of the patients ranged from 55 to 80 with a mean of 69·1 years. There were eleven males and three females. The time of documented photosensitivity prior to azathioprine treatment ranged from 3 to 150 months with a mean of 50·5 months. The duration of treatment was determined according to clinical response and ranged from 1·5 to 33 months with a mean of 11·5 months. All patients were seen and assessed monthly. Liver function tests as well as haemoglobin, white cell and platelet counts were carried out at each visit.

Case histories

Case 1. A 62-year-old man in 1966 developed eczema which became erythrodermic in 1975. A localization to the light-exposed areas was noted in 1978. Biopsy of the skin in 1976 showed chronic eczema but in 1978 changes suggestive of CAD were seen. Skin phototests carried out with an irradiation monochromator showed reduced MEDs and abnormally marked papular responses from 300 nm up to 400 nm in the UV-B and UV-A wavelength ranges. In February 1979, azathioprine was commenced in a dosage of 50 mg b.d. Improvement was noted within 6 weeks and he was able to go on holiday in August 1979 without the use of sunscreens. Azathioprine was discontinued after 12 months of treatment and no relapse was seen. He died of chronic obstructive airways disease in 1981.

Case 2. A 64-year-old male developed eczema in 1962 and photosensitivity was noted in 1965. In March 1980 he became erythrodermic with markedly accentuated changes on the face and on the arms and thighs up to the clothed sites. Skin biopsy showed chronic eczema, and phototests showed reduced MEDs and abnormally marked papular response at 300 and 307·5 nm in the UV-B wavelength range. Azathioprine 50 mg b.d. was commenced in June 1980 and by November 1980 his skin had cleared. In April 1981 he was able to holiday in Majorca. He was then maintained for several more months on treatment with 25 mg/day and remained in remission.

Case 3. A 60-year-old male developed slight hand eczema in 1957 but in 1976 marked involvement of face and hands was followed by generalized erythroderma. Biopsy showed changes of CAD and phototests showed reduced MEDs and abnormally marked papular responses from 300 nm up to 600 nm in the UV-B and UV-A wavelength ranges. He was

TABLE 1. Clinical details of fourteen patients with chronic actinic dermatitis treated with azathioprine

Age	Sex	Duration of disease prior to treatment (months)	Wavelengths of phototest abnormality (nm)	Patch (PT) and photopatch (PPT) test sensitivities	Duration of azathioprine treatment (months)	Daily azathioprine dose (mg)	Biopsy result	Outcome of treatment
72	M	83	300–400	Nil	15	150	CAD	Cleared completely. Treatment stopped. Died of cerebrovascular disease
71	F	36	300–307·5	Nil	10	100	CAD	Cleared completely. Treatment stopped. No recurrence in summer
74	F	36	300–307·5	PT: balsam of Peru, chrysanthemum, thiuram	9	150	not done	Cleared completely within 2 months. Treatment stopped
55	M	3	300–360	PT: neomycin PPT: nil	1·5	100	not done	Cleared completely within 6 weeks. Treatment stopped
64	M	180	300–307·5	Nil	18	100	CAD	Cleared completely. Treatment stopped
62	M	40	300–400	Nil	12	100	CAD	Much improved within 2 months. Treatment stopped. Died of obstructive airways disease
72	M	84	300–400	Nil	15	200	CAD	Improved in 8 months. Died of heart disease
60	F	36	360	PT: balsam of Peru, thiuram, colophony PPT: nil	13	100	CAD	Improved slowly
60	M	24	360	Nil	24+7	200	CAD	Cleared completely in 24 months. Relapsed over 10 months. Recommenced treatment. Improved over 7 months
80	M	60	360	PT: chrysanthemum, thiuram PPT: nil	24	100	CAD	Improved over 6 months. Relapsed despite continuing treatment
74	M	36	340	PT: wool alcohols PPT: nil	2	150	CAD	Stopped treatment—severe diarrhoea and vomiting
60	M	36	360	PT: cobalt, colophony, cresol PPT: nil	1	100	CAD	Stopped treatment—severe diarrhoea and vomiting
75	M	24	600	Nil	6	200	CAD	No benefit
61	M	30	500	Nil	6	150	CAD	No benefit

commenced on azathioprine in February 1978 in a dose of 50 mg q.d.s. His skin then cleared and remained so throughout the summer of 1979. Treatment was therefore discontinued in February 1980 but he relapsed over the next 10 months. Treatment was recommenced in April 1981 and improvement was again noted.

RESULTS

Nine of the fourteen patients reviewed benefited, apparently permanently, from treatment with azathioprine (Table 1). One further patient cleared initially but then relapsed while still on treatment. Two other patients showed no improvement and the remaining two discontinued therapy because of gastrointestinal upset, namely abdominal discomfort, diarrhoea and vomiting.

DISCUSSION

Although no absolute conclusion can be reached concerning the effects of azathioprine treatment in CAD since occasional spontaneous improvements are known to occur in the condition (Hawk & Magnus, 1978), our results are so encouraging that we feel that a double-blind controlled trial is justified. Many patients appear to remain clear after discontinuing the therapy. This may reflect spontaneous remission as mentioned above but is very likely a prolonged effect of azathioprine. The mechanism of action of azathioprine in this condition is unknown, but it is interesting that there was no change in the skin phototests of four of the patients when they were repeated several months after improvement had occurred and therapy had ceased, whereas in the one documented case of spontaneous remission, the phototests had improved (Hawk & Magnus, 1978). The treatment was well tolerated in most cases but gastrointestinal side-effects necessitated discontinuation of therapy in two patients out of the fourteen. Haematological abnormalities were not a problem. Because of these excellent results in a previously intractable and socially devastating illness we intend to continue using azathioprine in severe selected cases of CAD while controlled studies of the effect of the therapy are in progress.

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Effect of low dose cyproterone acetate on the response of acne to isotretinoin

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SUMMARY

Twenty-seven males with severe acne were treated for 12 weeks with 0·05 mg/kg/day isotretinoin (ten patients) or 5 mg daily cyproterone acetate (eight patients) or both drugs together in these doses (nine patients). With isotretinoin, the sebum excretion rate (SER) fell by 45% ± 9% s.e.m. ($P < 0{\cdot}0025$), lesion count fell by 65% ± 10% ($P < 0{\cdot}0005$) and median clinical severity fell from 8 to 2·5 ($P = 0{\cdot}01$). With cyproterone acetate there was a 17% ± 12% (NS) fall in SER, a 15% ± 10% (NS) fall in lesion count and the median severity was unchanged. Patients treated with both drugs showed a 42% ± 13% reduction in SER ($P < 0{\cdot}005$), a 68% ± 11% decrease in lesion count ($P < 0{\cdot}0005$) and a decrease in median severity from 8 to 4 ($P < 0{\cdot}01$) which was no different from the response to isotretinoin alone. Isotretinoin increased serum cholesterol from 4·4 mmol/l ± 0·3 s.e.m. to 4·7 mmol/l ± 0·3 s.e.m. ($P < 0{\cdot}01$), serum triglyceride from 0·73 mmol/l ± 0·07 s.e.m. to 0·96 mmol/l ± 0·14 s.e.m. ($P < 0{\cdot}05$) and γ-glutamyltransferase (GGT) from 15·9 i.u./l ± 2·1 s.e.m. to 19·0 i.u./l ± 2·4 s.e.m. ($P < 0{\cdot}01$). Comparison of the area under the concentration-time curve for triglyceride and high density lipoprotein (HDL)-cholesterol showed that the changes were smaller when isotretinoin was combined with cyproterone acetate. We conclude that the effect of isotretinoin in acne was not enhanced by the antiandrogen, but the increase in serum triglyceride and decrease in HDL-cholesterol produced by the retinoid were reduced by combination with the antiandrogen.

Isotretinoin and cyproterone acetate (CPA) are both effective treatments for acne, we presume by virtue of their inhibition of sebum excretion. However, isotretinoin causes changes in serum lipids and liver function tests at the doses of 0·5–1·0 mg/kg/day used most frequently (Lyons *et al.*, 1982), and CPA has antiandrogenic side-effects such as reduced libido at the doses of 25–100 mg/day that we have found effective (Marsden, Shuster & Lyons, 1984). Since isotretinoin and CPA inhibit sebaceous glands by different mechanisms (Marsden, Shuster & Lyons, 1984) it seemed possible that the two drugs might act synergistically, so that given together low doses of each might still be effective, thus minimizing side-effects. We therefore measured the response to isotretinoin and CPA alone and in combination.

METHODS

Twenty-seven males with severe acne were included in the study to which all gave informed consent. Ten men aged 17–28 years were treated with 0·05 mg/kg/day isotretinoin, eight men aged 18–28 years were treated with 5 mg CPA daily and nine men aged 18–27 years were treated with 0·05 mg/kg/day 13-*cis*-retinoic acid (isotretinoin) plus 5 mg daily CPA. In all cases treatment was for 12 weeks.

Sebum excretion rate (SER), total acne lesion count and severity of acne on a 0–10 analogue scale were measured before treatment and at 4-weekly intervals until 1 month after treatment had ended. The following biochemical measurements were made at the same intervals in patients receiving isotretinoin alone or combined with CPA: fasting levels of serum cholesterol, triglyceride and HDL-cholesterol, concentrations of γ-glutamyltransferase (GGT), serum albumin and total protein, and liver function tests (bilirubin, alkaline phosphatase, aspartate aminotransferase). Side-effects during treatment were also recorded. All statistical comparisons were by Student's *t*-test except for severity of acne, which was compared by the Wilcoxon paired rank sum test.

RESULTS

Treatment with isotretinoin alone produced its largest reduction in SER by 4 weeks, from 1·8 μg/cm^2/min ± 0·2 s.e.m. to 0·7 μg/cm^2/min ± 0·1 ($P < 0{\cdot}0025$). SER remained at about this level, being reduced from the pretreatment level by 45% ± 9% after 12 weeks treatment ($P < 0{\cdot}0025$, Fig. 1). Cyproterone acetate however, produced only a small decrease in SER from 1·7 μm/cm^2/min ± 0·2 s.e.m. before treatment to 1·4 μg/cm^2/min ± 0·1 after 12 weeks (17% ± 12%) but none of the values at 4, 8 or 12 weeks differed significantly from the pretreatment level (Fig.

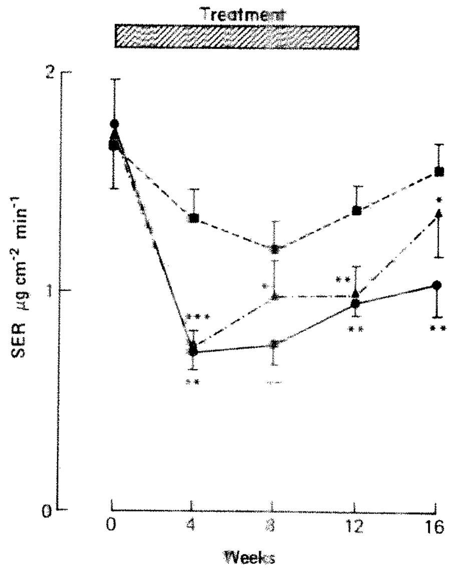

FIGURE 1. Decrease in sebum excretion rate (SER) before, during and after 12 weeks treatment with isotretinoin (●), CPA (■), and isotretinoin combined with CPA (▲). $^*P < 0{\cdot}01$, $^{**}P < 0{\cdot}005$, $^{***}P < 0{\cdot}001$.

1). Combination of 0·05 mg/kg/day isotretinoin with 5 mg/day CPA produced a similar decrease in SER to that produced by isotretinoin alone, maximal at 4 weeks, when it had decreased from 1·7 µg/cm^2/min ± 0·2 s.e.m. to 0·7 µg/cm^2/min ± 0·1 ($P < 0\cdot001$). At 12 weeks SER was reduced by 42% ± 13% ($P < 0\cdot005$, Fig. 1).

Acne lesion counts fell progressively during the treatment period with isotretinoin alone, with a maximum decrease after 12 weeks from 73 ± 9 s.e.m. to 25 ± 3 or 65% ± 10% ($P < 0\cdot0005$, Fig. 2). With CPA alone there was only a small, non-significant reduction in lesion count from 66 ± 8 s.e.m. to 57 ± 9 (15% ± 10%). Treatment with both drugs produced a decrease in lesion count, maximum at 12 weeks, from 125 ± 13 s.e.m. to 42 ± 9, or 68% ± 11% ($P < 0\cdot0005$), which was no different from that seen after isotretinoin alone.

The grade of severity of acne fell from a median of 8 before treatment with isotretinoin to 2·5 after 12 weeks ($P < 0\cdot01$, Fig. 3). With CPA, there was no change in median severity, and with isotretinoin and CPA combined, median severity fell from 8 before treatment to 4 at the end of treatment ($P < 0\cdot01$), similar to the decrease after isotretinoin alone.

Treatment with isotretinoin increased concentrations of serum cholesterol from a pretreatment level of 4·4 mmol/l ± 0·3 s.e.m. to 4·7 mmol/l ± 0·3 after 12 weeks ($P < 0\cdot01$) (Fig. 4) and serum triglyceride levels increased from 0·73 mmol/l ± 0·07 s.e.m. to 0·96 mmol/l ± 0·14 ($P < 0\cdot05$). Levels of HDL-cholesterol fell during treatment from 1·09 mmol/l ± 0·06 s.e.m. to 1·03 mmol/l ± 0·06, but this was not significant ($t = 1\cdot5$, $P < 0\cdot1$), although values at the end of treatment were lower in eight out of ten patients. Gamma-glutamyltransferase increased steadily throughout treatment from 15·9 i.u./l ± 2·1 s.e.m. to 19·1 i.u./l ± 2·4 ($P < 0\cdot005$). The changes in these measurements during treatment with isotretinoin combined with CPA (Fig. 4) are compared with those for isotretinoin alone by computing the area under the concentration–time curve (AUC) between 0 and 12 weeks, using the concentration at 0 weeks as zero. No difference was found between mean cholesterol AUC for isotretinoin alone (1·6 ± 0·8 s.e.m.) and

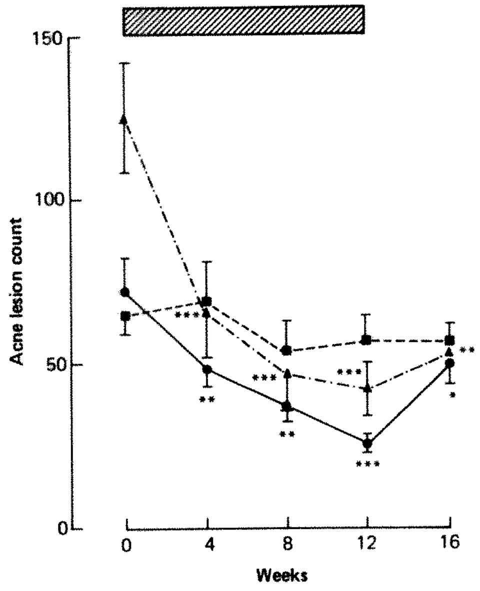

FIGURE 2. Change in acne lesion count before, during and after 12 weeks treatment with isotretinoin (●), CPA (■), and isotretinoin combined with CPA (▲). $^*P < 0\cdot0125$, $^{**}P < 0\cdot005$, $^{***}P < 0\cdot001$.

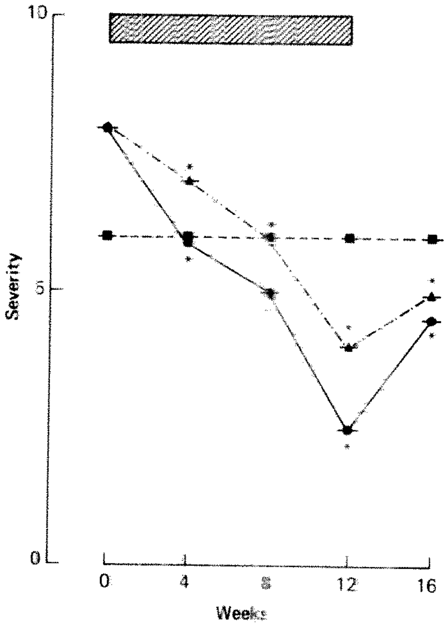

FIGURE 3. Change in acne severity before, during and after 12 weeks treatment with isotretinoin (●), CPA (■), and isotretinoin combined with CPA (▲). $^*P < 0{\cdot}01$.

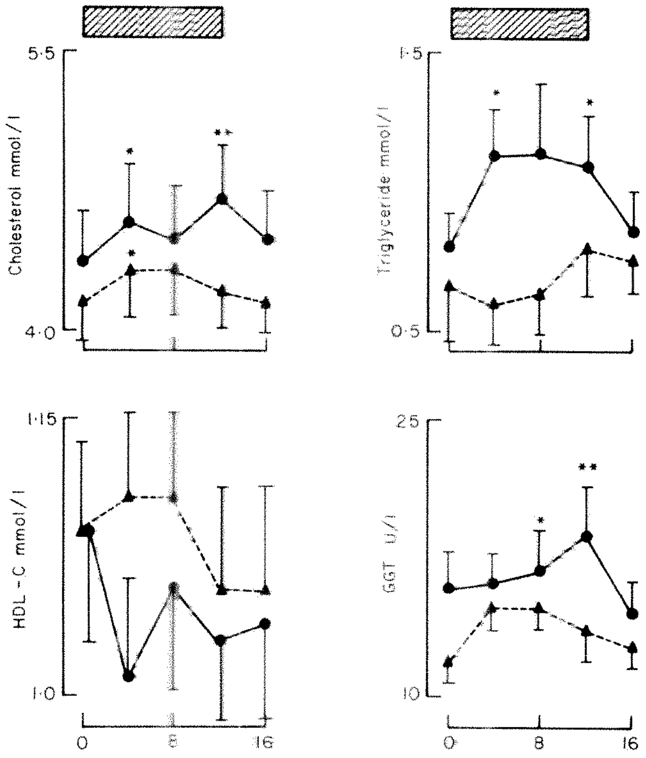

FIGURE 4. Changes in serum cholesterol, triglyceride, HDL-cholesterol (HDL-C) and γ-glutamyl transferase (GGT) before, during and after 12 weeks treatment with isotretinoin (●), and isotretinoin combined with CPA (▲). $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

for isotretinoin with CPA (1·5 ± 0·9 s.e.m.). However, mean triglyceride AUC was 2·26 ± 1·02 s.e.m. after isotretinoin, and −0·39 ± 0·98 s.e.m. after isotretinoin with CPA ($P < 0·05$). Although HDL-cholesterol AUC was greater with isotretinoin alone (−0·45 ± 0·29 s.e.m.) than with the combined drugs (0·11 ± 0·34 s.e.m.) this difference was not significant ($t = 1·32$, $P < 0·10$). However, GGT AUC was significantly lower with the retinoid alone (11·5 ± 3·3 s.e.m.) than with the combination (27·7 ± 7·5 s.e.m., $P < 0·05$). In all cases these changes had reversed 4 weeks after treatment had stopped, when they were not significantly different from pretreatment levels. No significant changes were found in levels of serum albumin, total protein, or in other liver function tests.

Isotretinoin was well tolerated although all patients except one taking it either alone, or combined with CPA, had cheilitis. Two patients on CPA alone noticed reduction in libido by 8 weeks of treatment. These side-effects had reversed within 8 weeks of treatment finishing. Two patients stopped treatment at 4 weeks because of failure to improve (one on CPA, one on both drugs). They were excluded from the trial.

DISCUSSION

The results show that 0·05 mg/kg/day isotretinoin produces a 45% decrease in SER after 12 weeks treatment which is similar to the findings of King *et al.* (1982) with 0·1 mg/kg/day isotretinoin. Previous findings show that with 0·8 mg/kg/day isotretinoin, SER was reduced by 90% after 12 weeks (Lyons, Marsden & Shuster, 1982). Cyproterone acetate 5 mg/day gave a small decrease in SER of 17%; after treatment with 25 mg/day CPA (Marsden, Shuster & Lyons, 1984) we found a reduction in SER of 39% and with 100 mg/day a reduction of 68%. Thus, 0·05 mg/kg/day isotretinoin appears to be on the centre of the linear part of the dose–response curve whilst 5 mg CPA is at the lower end. If synergy between CPA and isotretinoin occurred, the maximum therapeutically useful dose of CPA in males would be 5 mg daily as higher doses reduce sperm count and potency. However, when the two drugs were given in combination in these doses their effect was very similar to that found with isotretinoin alone, with a similar decrease in SER, lesion count, and severity of acne. Thus, despite the evidence that the two drugs inhibit sebum production by different mechanisms, we have found no evidence that they act synergistically.

The explanation for this lack of synergy may be that isotretinoin causes gross disturbance of sebaceous cell structure (Landthaler *et al.*, 1980) and function [shown by widespread changes in constituent sebaceous lipids, (Marsden, Shuster & Lyons, 1984)] and these changes may reduce the effect of androgen, and indirectly therefore of antiandrogen. To test this the dose of CPA would have to be increased in proportion to the inhibitory effect of isotretinoin on the sebaceous gland.

Previous work has shown that isotretinoin in a dose of 0·8 mg/kg/day produces significant increases in serum cholesterol and triglyceride, and decreases in HDL-cholesterol (Lyons *et al.*, 1982). Gamma-glutamyltransferase also increased, and there was a small decrease in serum albumin concentration. Our findings with a dose of only 0·05 mg/kg/day show very similar changes, except that we found no difference in serum albumin. The biological effects of these changes are unlikely to be of much consequence during therapy for only 12 weeks (Kingston, 1983) although their importance may be greater with longer periods of treatment. However, they cannot be avoided by further reduction in dosage as a dose of less than 0·05 mg/kg/day has too small an effect on SER to be therapeutically useful. In this respect the much smaller changes in AUC for triglyceride and serum HDL-cholesterol after treatment with isotretinoin and CPA combined is promising. As the effect of CPA on serum lipids was not measured in this study, it is unclear whether the results are due to an effect of CPA alone on serum lipids (Moltz *et al.*, 1980; Damgaard-Pedersen & Fogh, 1980), or to a prevention of the changes caused by isotretinoin. Although two patients noticed reduced libido during CPA therapy, this was not severe, and it reversed when treatment was stopped. No patients had impotence. In conclusion, although we found no evidence of synergy of effect of isotretinoin and CPA, the findings suggest that the addition of CPA may be useful by ameliorating the unwanted effect of isotretinoin on serum lipids. This should be confirmed before combined treatment can be recommended.

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Short-contact dithranol therapy—a comparison with the Ingram regime

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SUMMARY

We have compared a short-contact dithranol regime with the traditional Ingram regime in the treatment of forty-three patients with plaque psoriasis. The Ingram regime produced a significantly faster rate of improvement of the psoriasis than the short-contact regime. The degree of relapse 12 weeks after stopping treatment was identical for both regimes. The irritant effect was more troublesome with short-contact dithranol therapy. Nevertheless, short-contact dithranol regimes may have a place in home treatment.

The Ingram regime (Ingram, 1953) of daily tar baths, ultraviolet radiation (UV-B) and dithranol in hard Lassar's paste applied under stockinette dressings has been used for the treatment of plaque psoriasis for almost 30 years. It is both effective and safe; however, the complexity of the regime and its messiness have restricted its use for therapy in the patient's home. These drawbacks have led to modifications; for example, Seville (1975) showed that dithranol was the effective component of the Ingram regime and that tar baths and ultraviolet radiation played only a minor role in most patients. Further modifications to the dithranol formulation led to the appearance of proprietary dithranol preparations, e.g. Dithrocream® (Dermal) and Psoradrate® (Eaton), which were easier to apply and more cosmetically acceptable than dithranol in Lassar's paste, but the low concentration of dithranol has limited the effectiveness of these newer formulations for many patients.

Studies of the penetration of radiolabelled dithranol into cadaver epidermis led Schaefer *et al.* (1980) to conclude that after removal of the stratum corneum most dithranol penetrates the epidermis within 30 minutes of application. They compared this situation to the disturbance of the stratum corneum in psoriasis. This suggested that the removal of dithranol from a psoriatic plaque after 30 minutes would be effective and limit the irritation of the surrounding normal skin. Following this work, Runne and Kunze (1982) performed a comparison between a 10–12 minute application of dithranol (short-contact dithranol therapy) and a 24-hour application in the treatment of psoriasis. Both schedules involved the application of dithranol and salicylic acid in a base of soft paraffin. The mean clearance time was 6·8 days less with the short-contact

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regime. However, the design of this trial may be criticized for two reasons: firstly, the initial concentration of dithranol was 10 times greater on the lesions treated by short-contact therapy; secondly, the irritancy of a 24-hour application of dithranol in a soft paraffin base may have limited the concentration of dithranol applied to the lesions.

We therefore performed a comparative study between short-contact dithranol therapy, as performed by Runne and Kunze (1982), and the Ingram regime.

METHODS

Forty-three consecutive patients with plaque psoriasis were randomly allocated to receive treatment by the Ingram regime or by short-contact dithranol therapy (Table 1). In both regimes the initial dithranol concentration was 0·5% and was increased through 1, 2 and 3% on alternate days according to tolerance. Stockinette dressings were applied in patients undergoing treatment by the Ingram regime, whereas no dressings were used in the group treated by short-contact therapy. All patients were treated daily by the nursing staff in our out-patient department.

Assessment was performed initially on day 0, then on days 7, 14, 21 and 28. Treatment was continued until clearance of psoriasis and the patients were reassessed 12 weeks after clearance to determine the degree of relapse.

Assessment was performed using the Psoriasis Area and Severity Index (P.A.S.I.) as described by Fredrickson and Petterson (1978). Scalp psoriasis was not treated as a part of this trial and the calculations for involvement of the head were omitted from the formula. The trunk, upper limbs and lower limbs were assessed separately for each of the following variables:

Percentage area of involvement	(0–6 scale)
Palpability of lesions	(0–4 scale)
Erythema of lesions	
Scaling of lesions	

The irritant effect on the uninvolved skin was also assessed on a 0–4 scale. The same observer

TABLE 1. Treatment schedules for the Ingram regime and short-contact therapy

Ingram regime	Short-contact therapy
Daily tar bath	No tar bath
Ultraviolet radiation	No ultraviolet radiation
Dithranol in hard Lassar's paste*	Dithranol + 5% salicylic acid in soft paraffin
Stockinette dressing	No dressings
Remove paste after 24 hours with arachis oil	Remove after 10–20 minutes (according to tolerance) by thorough washing with soap and water

* Formula: Zinc oxide 24%, starch 24%, hard paraffin 3%, salicylic acid 2%, yellow soft paraffin to 100%.

performed the assessment at each visit. The P.A.S.I. scores at each assessment were then calculated according to the formula:

$$\text{P.A.S.I.} = \underbrace{0{\cdot}3A(E+P+S)}_{\text{Trunk}} + \underbrace{0{\cdot}2A(E+P+S)}_{\text{Upper limbs}} + \underbrace{0{\cdot}4A(E+P+S)}_{\text{Lower limbs}}$$

where 0·3, 0·2 and 0·4 represent the relative proportion of the body surface area on the trunk, upper limbs and lower limbs respectively and E, P, S and A represent erythema, palpability, scaling and area.

RESULTS

Thirty-eight patients completed the study, twenty treated by short-contact therapy and eighteen by the Ingram regime. Four patients were withdrawn from the Ingram regime (two patients defaulted from treatment, one patient could not tolerate dithranol, even at a low concentration, and one patient's psoriasis failed to clear). One patient was withdrawn from short-contact therapy due to failure to clear the psoriasis.

The results for the remaining thirty-eight patients are shown in Figure 1. There was no significant difference in mean P.A.S.I. score between the groups at the initial visit (group *t*-test). After 1 week of treatment the group treated by the Ingram regime showed a significantly greater reduction of the P.A.S.I. score ($P < 0{\cdot}001$) than the short-contact therapy group. These differences were maintained on days 14 and 21 but there was no significant difference at the follow-up visit (FU) 12 weeks after completing therapy.

Omitting the area of involvement (A) from the calculations and plotting the sum of the severity scores (E + P + S) at each visit (Fig. 2) the differences between the two groups during treatment were even more apparent. Two factors explain this apparent discrepancy: firstly, the area of involvement is the slowest feature to show improvement, and secondly, it is the most difficult to assess clinically.

Table 2 shows the number of patients with varying percentage reductions in the P.A.S.I.

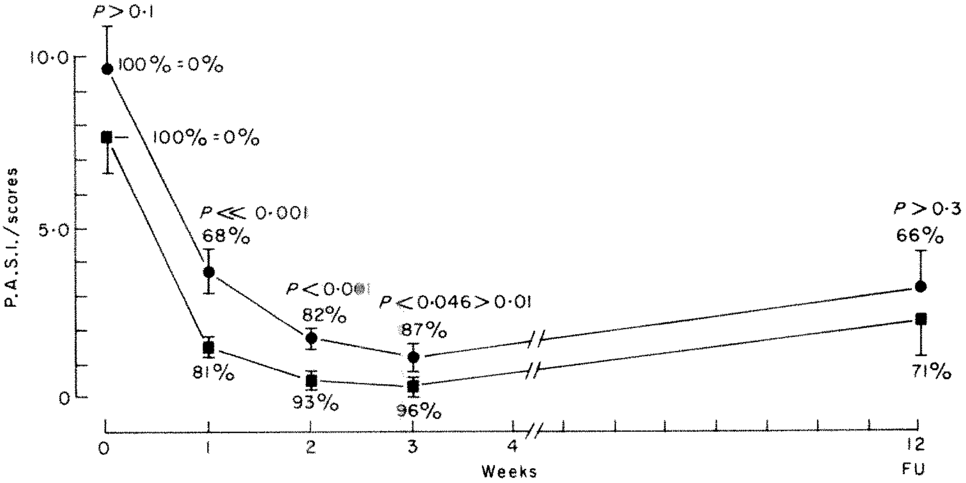

FIGURE 1. Mean P.A.S.I. score and percentage reduction (compared with the initial score) at each assessment. (●) Short-contact therapy, $n = 20$. (■) Standard therapy, $n = 18$.

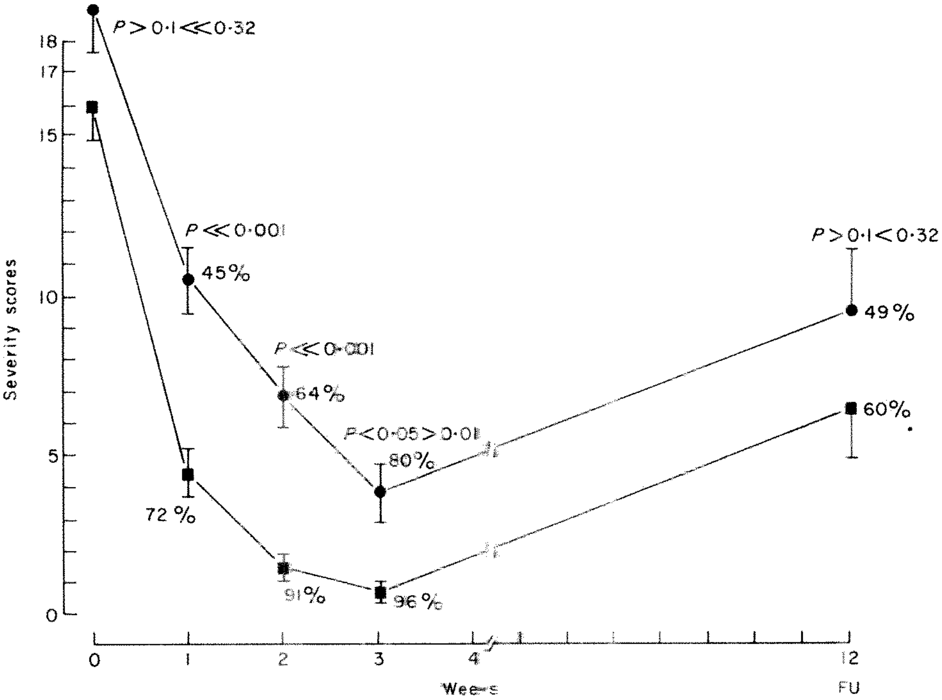

FIGURE 2. Mean severity scores (E + P + S) and percentage reduction (compared with the initial score) at each assessment. (●) Short-contact therapy, $n = 20$. (■) Standard therapy, $n = 18$.

TABLE 2. Number of patients achieving various percentages of reduction in P.A.S.I. after 3 weeks of therapy

Percentage reduction	100	90+	80+	70+	60+	Total number
Short contact	8	4	3	3	2	20
Ingram	10	6	1	0	1	18

score after 3 weeks of therapy. It can be seen that sixteen out of eighteen patients treated by the Ingram regime were improved by at least 90% after 3 weeks, compared with twelve out of twenty patients treated by short-contact therapy. This trend in favour of Ingram therapy is not statistically significant (Exact Probability test).

The irritant effect of dithranol on the uninvolved skin was more marked in the group treated by short-contact therapy (Fig. 3).

Although the staining of the skin was not formally assessed, the staining was much less marked with short-contact therapy.

DISCUSSION

Our results clearly show that both treatment schedules are effective in clearing psoriasis and

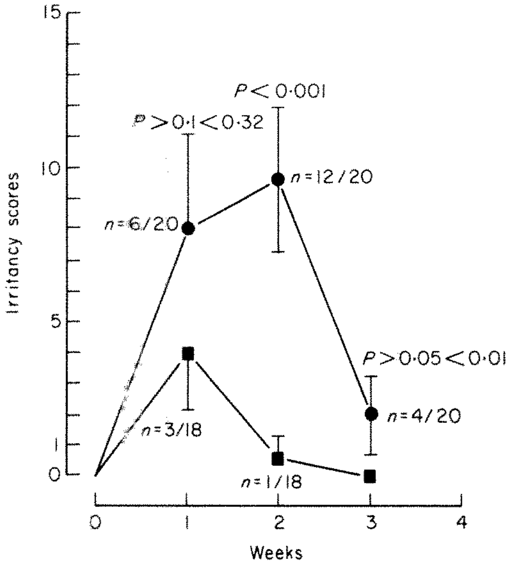

FIGURE 3. Sums of irritancy scores and the number of patients with an irritant reaction at each assessment. (●) Short-contact therapy. (■) Standard therapy.

there is no difference in the degree of relapse of either treatment 12 weeks after completing therapy. The Ingram regime produces a more rapid improvement and is therefore the treatment of choice for those patients with severe psoriasis who require either in-patient or out-patient treatment at hospital. The slower rate of improvement may not be as crucial for patients undergoing treatment at home and the lack of messy applications after the treatment period and the absence of dressings would seem to make this an attractive alternative to hospital treatment. However, the marked irritant effects experienced by several patients treated by short-contact therapy make us unable to recommend the regime in its present form for home use. Marsden *et al.* (1983) also considered that the increased burning of short-contact therapy was likely to limit its usefulness. These authors differ from us in finding no difference in the speed of clinical clearing between short-contact and conventional dithranol therapy.

The irritant effects of short-contact therapy are almost certainly due to the formulation of the dithranol in a soft paraffin base which proved exceedingly difficult to remove by washing thoroughly in soap and water. In several cases the irritant effects were seen several centimetres away from a psoriatic plaque, suggesting that spreading of the dithranol had occurred during the washing process. We would have preferred to have used an alternative method for removing the dithranol preparation but we were following the Runne and Kunze (1982) regime exactly. In the Ingram regime the hard Lassar's paste base does not melt under normal circumstances and therefore localizes the dithranol to the lesion. Moreover the paste is easily removed by arachis oil on cotton wool.

It remains an attractive goal to aim for a safe and effective home treatment of psoriasis; this would benefit many of the well-motivated patients who attend our clinics with less severe psoriasis and many of the patients who have been deterred from seeking treatment of their psoriasis due to the lack of a simple and effective treatment. We are continuing to study various modifications of short-contact therapy to assess the most practical regime for home use.

Further support for the use of short-contact dithranol therapy at home comes from a recent report by Donaldson and Cunliffe (1983) who found a modified regime to be a useful remedy in well-motivated patients with uncomplicated psoriasis.

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Case Reports

A serum inhibitor of neutrophil chemotaxis associated with hyperglobulinaemia E in a patient with lymphoma, and recurrent skin infection

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SUMMARY

A patient with a strong serum inhibitor of neutrophil chemotaxis, a possible chemotactic factor inactivator and hyperimmunoglobulinaemia E is described. The patient had a persistent chronic dermatitis which appeared to develop following radiation of the central nervous system for an unclassified lymphoma. Other abnormalities included a general increase of serum immunoglobulins, impaired lymphocyte response to mitogens, and a high Epstein–Barr antibody titre. This case illustrates that an increased IgE level, previously reported in association with a cellular chemotactic defect, may coexist with serum inhibitors of neutrophil chemotaxis.

Chemotaxis, or the ability of polymorphonuclear neutrophils (PMNs) to move in a directed manner to gradients of chemotactic agents, is important in host defence against invasive bacteria and fungi (Ward, 1974; Ward, Johnson & Kreutzer, 1977; Quie & Cates, 1977; Becker, 1975; Gallin & Wolff, 1975; Zigmond, 1978). Three types of chemotactic defects have been described (Ward, 1974, 1976; Ward *et al.*, 1977; Gallin & Wolff, 1975): an inhibitor in serum acting directly on PMNs, a serum chemotactic factor inactivator (CFI) in which complement-derived leukotactic factor C5a becomes ineffective, and a cellular hyporesponsiveness.

Serum inhibitors of PMNs have been described in the Wiskott–Aldrich syndrome, IgA myeloma, malignancy, severe eczema and chronic mucocutaneous candidosis (Quie & Cates, 1977; Smith *et al.*, 1972). In one patient with recurrent infection, the chemotactic inhibitor was reported to be an IgG (Kramer, Perez & Goldstein, 1980). Serum IgE was not increased in any of these cases.

Increased levels of chemotactic factor inactivator have been reported in patients with Hodgkin's disease (Ward & Berenberg, 1974). Cellular neutrophil chemotaxis defect has been

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described in patients with infections and with hyperimmunoglobulinaemia E (Quie & Cates, 1977; Church *et al.*, 1976; Van Scoy *et al.*, 1975; Hill & Quie, 1974, 1975; Pincus *et al.*, 1975; DeCree *et al.*, 1978).

The patient reported here is unique in that she represents a combination of increased IgE, chemotactic serum inhibitor, possible CFI and lymphoma, without any defect in polymorphonuclear leukocytes.

CASE REPORT

A 36-year-old Caucasian woman developed muscular weakness, paraesthesiae, and generalized lymphadenopathy in April 1975. Investigations revealed a total white count of 3900/mm^3 with 37% neutrophils, 56% lymphocytes, 6% eosinophils, and 1% monocytes. Haemoglobin was 13·8 g %, and the platelet count was 145,000/μl. Bone marrow biopsies on two occasions were reported to show 'lymphocytosis'. Inguinal and cervical node biopsies were reported as 'hyperplasia with atypia'. Cerebrospinal fluid contained 35 mg/dl protein, 68 mg/dl glucose, negative serology, and 23 white blood cells per μl, mostly lymphocytes. The patient was considered to have an unclassified lymphoproliferative disease and was treated with prednisone 30 mg daily and isoniazid. This treatment improved her neurological symptoms and decreased her lymphadenopathy. In 4 months, medication was tapered off. In March 1976, the lymphadenopathy recurred and hepatosplenomegaly was noted. She was restarted on the same therapy, which was continued until November 1977. In January 1978, the patient was diagnosed as 'reticulum cell sarcoma, predominantly lymphocytic', based on the finding of abnormal cells in the cerebrospinal fluid. She received radiotherapy consisting of 4600 rad (cGy) to the whole spinal axis and 3500 rad (cGy) to the brain, resulting in marked improvement in the neurological symptoms and lymphadenopathy. In July 1979 she developed extensive dermatitis with erythema, papules, pustules, and crusted impetiginized lesions involving face, ears, axillae, inguinal, pubic areas, and extremities. At times, the skin had the appearance of atopic dermatitis, but did not respond to topical steroid therapy. *Staphylococcus aureus* coagulase-positive was isolated from the skin lesions. This problem has continued until the present time despite repeated antibiotic therapy. There was no hepatosplenomegaly or lymphadenopathy at this time.

Intradermal skin tests were negative for PPD, candida, and trichophytin. Laboratory studies revealed: albumin 4 g/dl, globulin 4 g/dl, IgG 3300 mg% (normal 500–1800 mg%); IgA 620 mg% (normal 200–300 mg%); IgM 161 mg% (normal 50–150 mg%), and IgE 4000 i.u. (normal 150–300 i.u.). Epstein–Barr virus capsid antibody was twice found in a high titre of 1:40,960 (determined by Dr Arye Rubenstein of Albert Einstein College of Medicine).

PMN chemotaxis

Chemotaxis was carried out as described by Ward (1976a), with some modifications. Leukocyte suspensions were prepared from normal controls and the patient's heparinized venous blood. One ml of 6% dextran was added to 5 ml blood. After 15–20 minutes of sedimentation, the leukocyte-rich plasma was separated, diluted with an equal volume of Hank's Balanced Salt Solution (HBSS) and centrifuged at 100 *g* for 3 minutes. The neutrophil sediment was washed in HBSS and resuspended to a concentration of 1×10^6/ml in HBSS. Patient's or normal serum was added to the PMN cell suspension at a concentration of 10%, and incubated at 37°C for 30 minutes on a shaker. The final cell suspensions added to the upper compartment of a modified Boyden Chamber (Neuroprope Corp., Bethesda, Md.) contained 10^6 neutrophils in 10% serum diluted in HBSS. Then 0·4 ml of these cell suspensions was added to the upper chambers. To generate complement-activated chemotactic factor, 1 ml of serum was incubated for 30 minutes with 20 mg of zymosan, and the supernatant was diluted 1:9 in HBSS. The lower chambers contained 0·25 ml of the 10% normal serum or zymosan-treated serum in HBSS. The chambers separated by 5 μm pore size membrane filters (Satorius GMBH-34 Gottingen, West Germany) were incubated at 37°C for 3 hours. The membranes were removed, fixed in absolute alcohol, and stained with haematoxylin. The lower surfaces of the stained membranes were examined under 400 × magnification. The total number of cells that had migrated to the bottom in ten random microscopic fields were counted and the results were expressed as the average of the ten fields. Duplicate chambers were used in each experiment.

RESULTS

Normal PMNs incubated in normal serum had a strong chemotactic response to zymosan-activated serum (Table 1), a mean of 62·2 cells migrating per high power field (hpf) compared with a mean of 4 cells migrating/hpf in a non-activated control serum (tests 1 and 2). A serum inhibitor was present when normal PMNs were incubated in the patient's serum. The chemotactic activity decreased to a mean of 12·5 cells/hpf (test 3).

A possible chemotactic factor inhibitor was demonstrated in test 4, where normal cells were incubated in normal serum, but the chemotactic agent was zymosan-activated patient's serum. There was a mean of 30 cells/hpf, compared with a mean of 62·2 cells/hpf in test 1 with zymosan-activated normal serum.

The patient's cells had no chemotactic defect, and this was shown in test 5. The patient's cells incubated in normal serum gave a chemotactic response of a mean of 53·8 cells/hpf compared with the control of 62·2. This test also showed that the serum inhibitor in the patient's serum had no cytotoxicity against the patient's cells. The chemotactic response was lowest (6 cells/hpf)

TABLE 1. Chemotaxis of patient's and normal donor's neutrophils (0·4 × 10⁶) in 10% serum in the upper chamber, and 10% serum or zymosan-activated serum in the lower chamber. Mean and standard deviation of migrated PMNs counts in replicated trials. Ten high power fields per trial

	Addition to Boyden Chamber		PMN migration to bottom of membrane		
Test	0·4 ml × 10⁶ PMNs Upper compartment	Zymosan generated chemotactant and normal serum Lower compartment	Mean	No. of trials	Standard deviation
1	Normal PMNs–Normal serum	Normal serum–Zymosan activated	62·2	9	8·3
2	Normal PMNs–Normal serum	Normal serum (not activated)	4·0	7	2·1
3	Normal PMNs–Patient's serum	Normal serum–Zymosan activated	12·5	9	7·5
4	Normal PMNs–Normal serum	Patient's serum–Zymosan activated	30·0	3	5·0
5	Patient's PMNs–Normal serum	Normal serum–Zymosan activated	53·8	4	11·3
6	Normal PMNs–Patient's serum	Patient's serum–Zymosan activated	6·0	2	2·8

Chemotaxis of normal cells (test 1) was inhibited by the patient's serum (test 3). The chemotactic activity of the zymosan-treated patient's serum (test 4) was less than that of the normal serum (test 1). The response was minimal when the patient's serum was used both in the upper chamber and the lower chamber for the generation of chemotactic factor (test 6). Test 2 is a negative control.

when the patient's serum was present in the upper chamber and her zymosan-treated serum was used in the lower chamber in test 6.

Figure 1 shows chemotaxis inhibition of normal PMNs by serially diluted patient's serum. No inhibitory activity was found in the dilution of the normal control serum. The patient's serum had no toxicity when tested against normal human lymphocytes.

Other neutrophil tests

The neutrophil adherence and nitroblue tetrazolium dye reduction tests were within normal limits. Adherence was 72% with a control of 80% (MacGregor, Spagnuolo & Lentner, 1974). Nitroblue tetrazolium assay on the patient's neutrophils gave an optical density of 0·49 compared with 0·45 for normal controls (Baehner & Nathan, 1968; Baehner, Boxer & Davis, 1976).

The patient's washed neutrophils gave a direct positive reaction with fluorescein-conjugated anti-human IgG but negative reactions with FITC-anti-IgE and FITC-anti-IgM (Verheugt *et al.*, 1977). Indirect immunofluorescence of the patient's serum was negative against normal PMNs using FITC anti-IgG, IgM and IgE. The patient's serum was negative when tested by agglutination against neutrophils of ten normal donors (Lalezari & Pryce, 1980). In mixed lymphocyte culture, the patient's cells were normally stimulated. The patient's lymphocytes showed a decreased response to pokeweed mitogen, 2553 c.p.m. (normal 12,026), and phytohaemagglutinin 5723 c.p.m. (normal 12,085) using [^{3}H]thymidine. Attempts to separate or remove the IgE fraction from the patient's serum were unsuccessful, using an immunoadsorption technique.

DISCUSSION

The patient described in this study had recurrent skin infection, a strong chemotaxis inhibitor in the serum and elevated serum IgE, but no defect in her neutrophils.

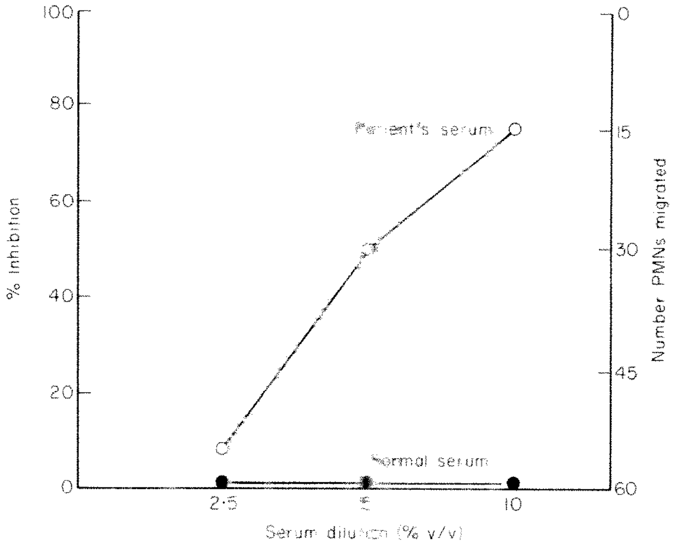

FIGURE 1. Chemotaxis inhibition in dilutions of patient's serum of 0·4 × 10^6 normal PMNs in upper compartment. Zymosan-activated normal serum in lower compartment. Control is dilutions of normal serum with normal PMNs.

Kramer *et al.* (1980) have described a serum chemotactic inhibitor consisting of an IgG immunoglobulin which they considered to be a PMN antibody that attached to a receptor on the surface of the PMN. Although the patient described here had no agglutinating neutrophil antibodies (Lalezari & Pryce, 1980) when tested against neutrophils of ten normal donors, a neutrophil autoantibody may have been present, since her neutrophils showed a direct positive immunofluorescence with fluorescein-conjugated anti-human IgG. Neutrophil specificity of this antibody is further confirmed by the finding that the serum showed no cytotoxicity against lymphocytes. The patient's PMNs functioned normally as measured by adherence (MacGregor *et al.*, 1974) and by nitroblue-tetrazolium dye reduction (Baehner & Nathan, 1968; Baehner *et al.*, 1976).

Ward and Berenberg (1974) have reported varying degrees of chemotactic factor inactivator in the sera of nine patients with Hodgkin's disease. Sera of their patients generated lower than normal chemotactic activity, as we apparently demonstrated in our patient (test 4). These authors isolated the CFI by a combination of salt precipitation, ion-exchange and gel filtration chromatography. The presence of CFI was shown by inactivation of known chemotactic agents.

The inhibition of chemotactic response of normal PMNs, observed in our patient when her zymosan-activated serum was placed in the lower chamber, may be due to seepage of her serum into the upper chamber. The possibility remains, however, that this effect was related to the presence of CFI.

Lymphocytes from patients with Hodgkin's disease may be defective in that they may show a failure to respond to mitogens (Ward & Berenberg, 1974). This patient's lymphocytes had a decreased response to pokeweed mitogen and phytohaemagglutinin. Goetzl and Rocklin (1978) have described a leukocyte inhibitory factor (LIF) due to a soluble product of activated normal lymphocytes which was an inhibitor of PMN chemotaxis. Since this patient's lymphocytes had a decreased response to pokeweed and PHA mitogens it is unlikely that they could be responsible for secreting a LIF-type lymphokine.

A striking finding was the marked increase of serum IgE. Defects in chemotaxis have been described in patients with recurrent skin infections, associated with hyperimmunoglobulinaemia E (Quie & Cates, 1977; Church *et al.*, 1976; Van Scoy *et al.*, 1975; Hill & Quie, 1974; Pincus *et al.*, 1975; DeCree *et al.*, 1978; Dahl, Greene & Quie, 1976). In all these reported cases, the chemotactic defects were cellular without serum inhibitors. This case differs in that the chemotactic defect was primarily due to a serum inhibitor (Table 1, test 3).

The role of IgE hypergammaglobulinaemia in the chemotactic defect is not clear. Hill and Quie have shown that histamine markedly depresses the chemotactic responses of normal human PMNs *in vitro*, and increased histamine release was present in sera of patients with hyperglobulinaemia E in addition to eczema, pruritus and urticarial rashes (Hill & Quie, 1975; Pincus *et al.*, 1975). The increase of histamine in patients with increased serum IgE is frequently associated with the allergic type of dermatitis, so that the increased serum histamine may be the consequence of the skin infections, in addition to, or in place of, the high IgE serum level. Defective chemotaxis in itself is followed by the spread and increased severity of the dermatitis which may result in further histamine release (Hill & Quie, 1974).

The chemotactic defects may also be related to humoral aberrations manifested by the elevation in serum IgG, IgA and IgM as well as IgE, and neutrophil autoantibodies. The cellular immunodeficiency in this patient may be due to the lymphoma (Ward & Berenberg, 1974) and irradiation (Rubin *et al.*, 1973; Order, 1977).

The high Epstein–Barr virus titre in this patient may be a marker of acquired T-cell immunodeficiency (Friedman-Kien *et al.*, 1982; Purtilo *et al.*, 1982) associated with lymphocyte malignancy.

Treatment for this patient's chemotactic defects has not been tried. Levamisole and ascorbic acid therapy have shown some promise (Rebora *et al.*, 1980).

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The morphology and hardness of the nail in two cases of congenital onychoheterotopia

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Two cases of congenital onychoheterotopia (ectopic nail) are described. In the first case electron microscopy showed spherical keratohyalin granules, and cytoplasmic desmosomes were seen in the keratinocytes of the nail matrix. The ectopic nail differed from the normal nail in the widening of the angle between the nail matrix and proximal nail fold, and in its radial growth to the skin surface. In the second case, clippings of the ectopic nail were taken and their hardness proved to be very similar to that of normal nails.

Two cases of congenital onychoheterotopia are described. In the first case we used light and transmission electron microscopy to study the nail morphology, and in the second case, the Vickers method was used to study the hardness of the ectopic nail.

CASE REPORTS

Case 1

A 71-year-old male had a small ectopic nail on the second toe of his right foot (Fig. 1). The ectopic nail was crescent-shaped, and it grew vertically upwards out of the toe. It had been present since birth, and the patient sometimes cut it because it was painful. The patient had been born with the second and third toes of his right foot webbed, and they had been surgically separated at the age of 3 years. X-ray examination revealed no abnormality in the bone of the right second toe. The ectopic nail was resected and examined by light and transmission electron microscopy.

Light microscopy. A horn-shaped mass of nail plate was found to continue down into the dermis (Fig. 2). With haematoxylin and eosin staining, the ectopic nail plate was a different colour from the surrounding stratum corneum. The left part of the nail plate was lighter and there was no granular layer, but the right part appeared dark, and the granular layer was clearly

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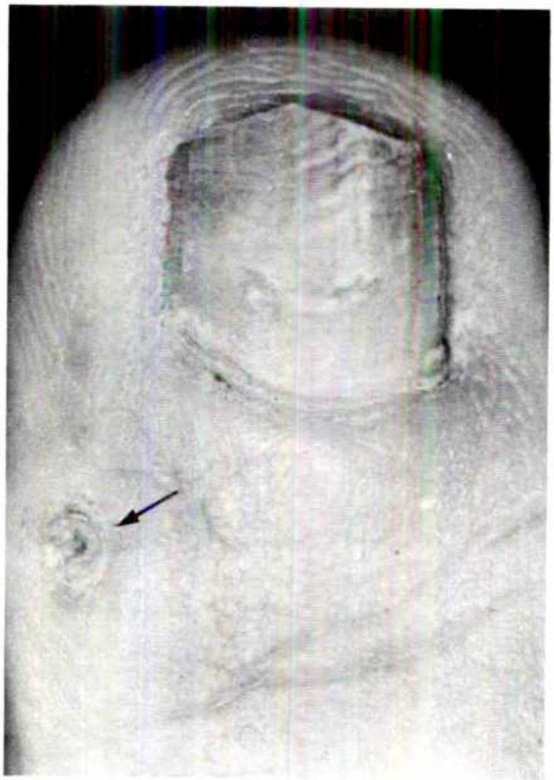

FIGURE 1. Case 1: The crescent-shaped nail plate of the ectopic nail, indicated by an arrow, was clearly visible on the right second toe.

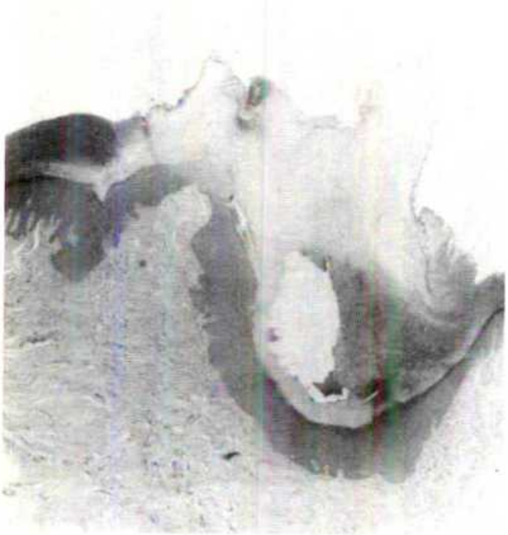

FIGURE 2. Case 1: Congenital onychoheterotopia seen by light microscopy.

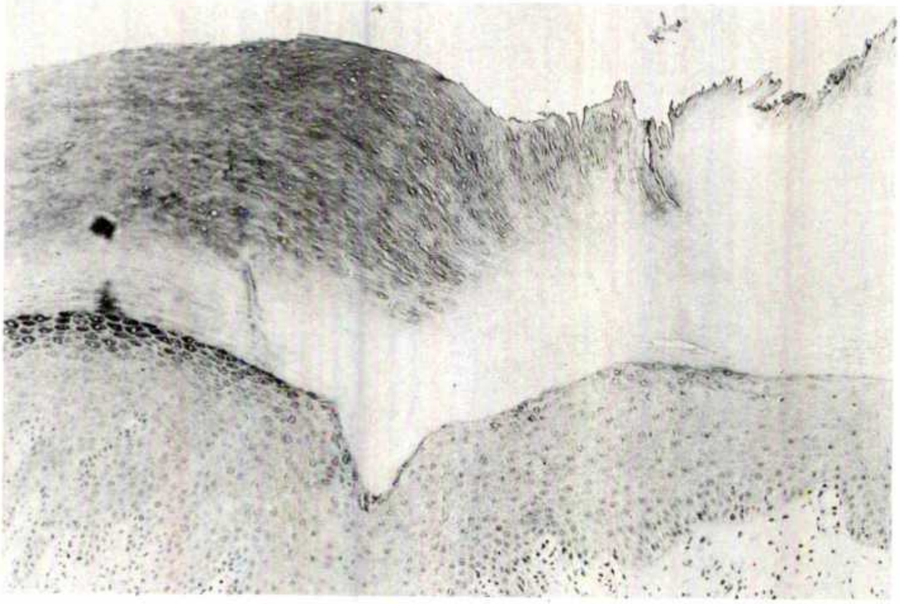

FIGURE 3. The border between the ectopic nail plate and surrounding epidermis. The nail plate is shown on the right, and no granular layer is visible.

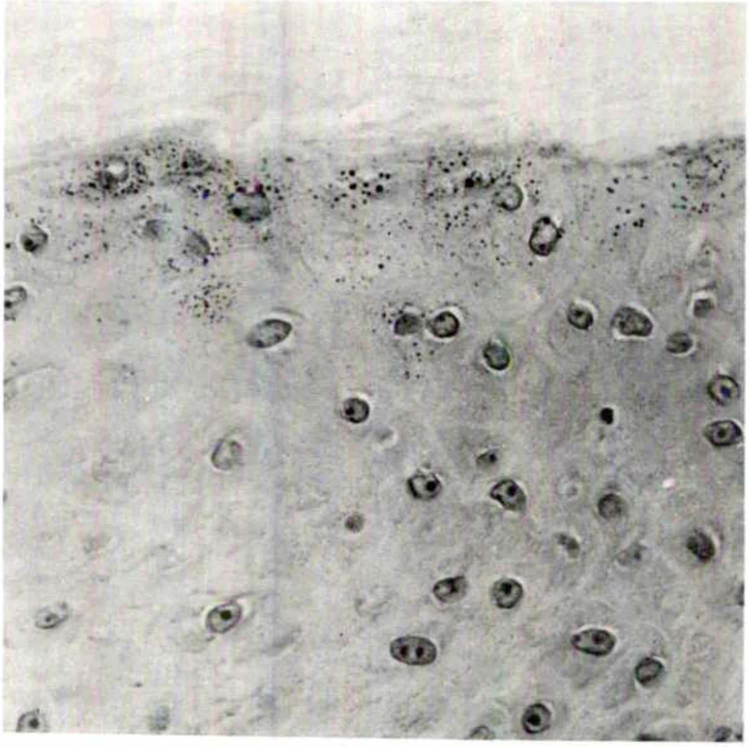

FIGURE 4. Higher magnification of the keratinocytes below the ectopic nail plate, showing spherical keratohyalin granules of various sizes in the cytoplasm.

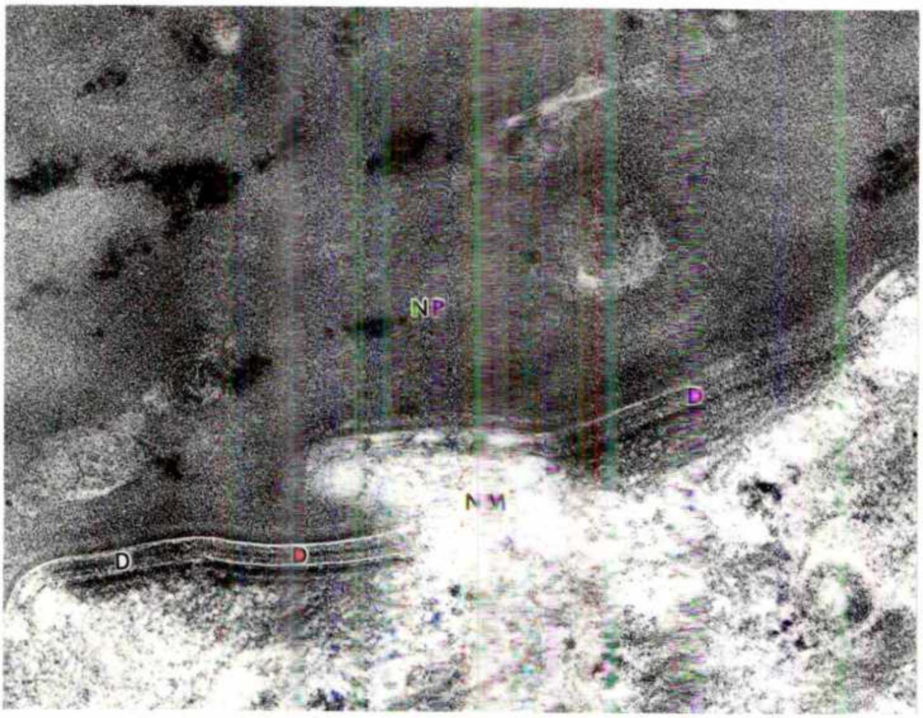

FIGURE 5. Electron microscopy. The ectopic nail plate (NP) appeared as a dark area, in which no keratin pattern could be seen. It was connected with the nail matrix (NM) by large desmosomes (D). (× 74,400.)

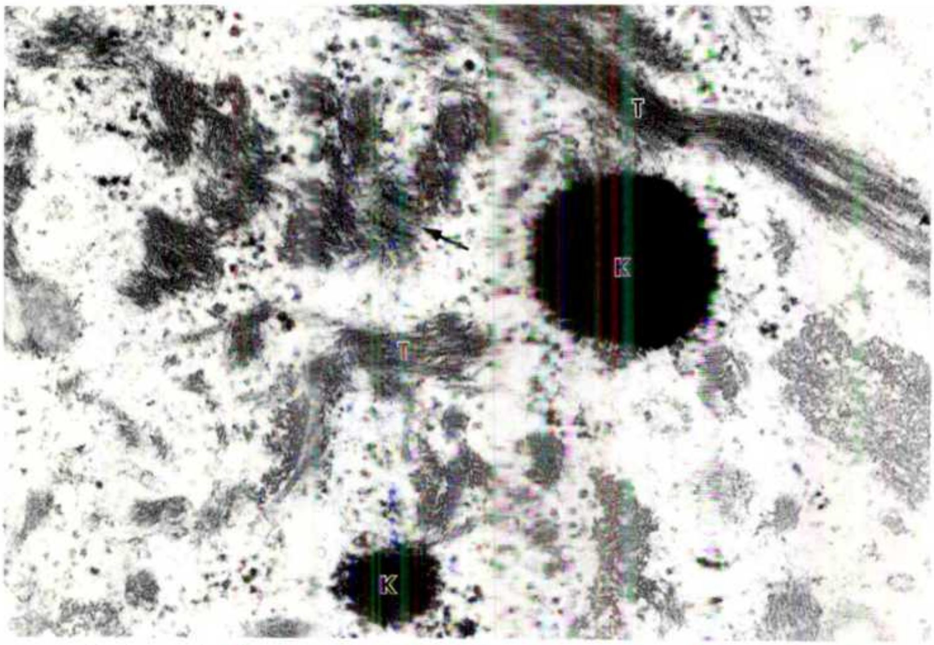

FIGURE 6. In the matrix cells, spherical keratohyalin granules (K) with numerous free ribosomes were observed. An intracytoplasmic desmosome is indicated by the arrow. T = tonofilament. (× 48,000.)

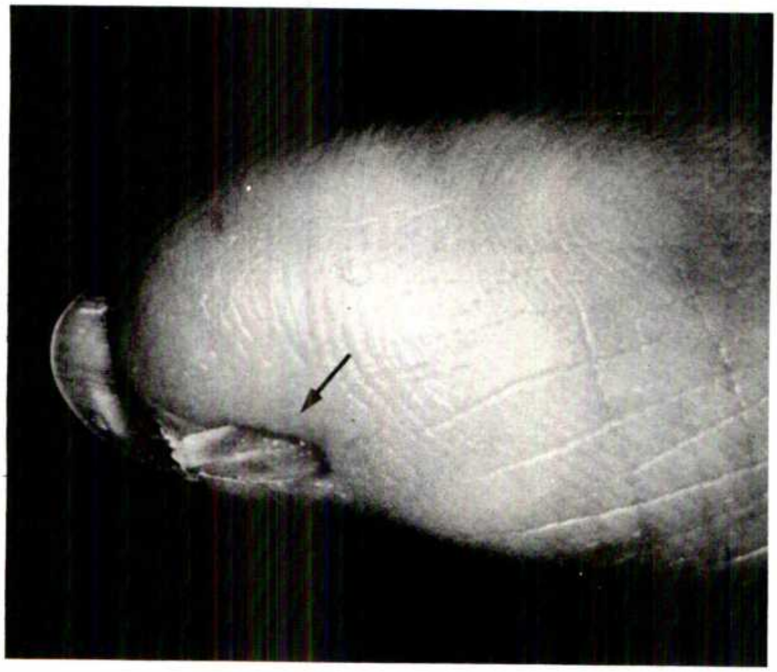

FIGURE 7. Case two: The small ectopic nail, indicated by an arrow, on the left fifth finger.

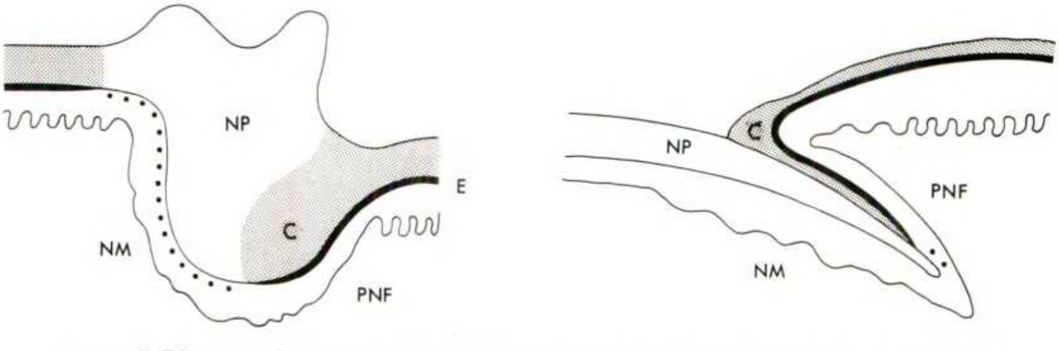

FIGURE 8. Diagrammatic comparison of congenital onychoheterotopia (left) and the normal nail (right). NP = nail plate, NM = nail matrix, C = cuticle, PNF = proximal nail fold, E = epidermis.

visible. A few dermal papillae could be seen under the nail plate. The epidermis around the nail plate showed hyperkeratosis and acanthosis. Figure 3 shows under higher magnification the left part of the nail plate continuing into the stratum corneum. The granular layer was not visible beneath the ectopic nail plate, whereas the granular layer can be clearly distinguished in the epidermis. Spherical keratohyalin granules of varying sizes were visible in the keratinocytes just below the nail plate (Fig. 4). Periodic acid–Schiff (PAS)-positive material present in this area was found to be amylase-digestible, indicating that it was glycogen. The glycogen looked like fine granules in suspension, almost as if they had been 'brushed on'.

Transmission electron microscopy. The dark area at the upper left of Figure 5 was the ectopic nail plate. No clear keratin pattern could be found in this area. Numerous large desmosomes were clearly visible (Fig. 5). Spherical keratohyalin granules and several tonofilaments were observed in the keratinocytes of the nail matrix. An intracytoplasmic desmosome was also clearly observed (Fig. 6).

Case 2

A 37-year-old female presented with a small congenital ectopic nail on the fifth finger of her left hand (Fig. 7). She sometimes cut the ectopic nail when it became too long. The hardness of a clipping from the ectopic nail was compared with that of nail clipping from the patient's fifth finger using the Vickers method. In this test a microdiamond pyramid is pressed on to the surface of the nail clipping at a fixed pressure and for a fixed period of time. After the pyramid is removed, the length of the impression left on the nail clipping is used to calculate the hardness of the nail. The ectopic nail clipping had a hardness of 20 on the Vickers scale, while the normal nail had a hardness of 23.

DISCUSSION

Congenital onychoheterotopia (ectopic nail) is extremely rare (Miura, 1978). Ectopic nails may look different from normal nails, since their matrices sometimes lie in an abnormal direction (Kikuchi, Ono & Ogata, 1978). In one of the two cases, the ectopic nail was on the patient's fifth finger, the commonest site for an ectopic nail (Zaias & Norton, 1980).

Our report seems to be the first to present clear light and transmission electron microscopic observations of congenital onychoheterotopia. In case 1, the ectopic nail plate was a different colour from the surrounding stratum corneum with haematoxylin and eosin staining, but the keratinocytes in the nail matrix had spherical keratohyalin granules similar to those keratinocytes located very near to the proximal nail fold in the normal nail matrix. This suggests that the ectopic nail bears a structural similarity to parts of the normal nail.

The glycogen granules in the keratinocytes of the ectopic nail matrix under PAS staining were similar to the fine glycogen granules found in psoriasis vulgaris (Montgomery, 1967), but they differed from the large spherical glycogen granules observed in trichilemmal keratinization (Winkelmann, 1967).

Based on these findings, a tentative model of the ectopic nail structure is shown (Fig. 8). In normal nails the angle formed by the nail matrix and proximal nail fold is wedge-shaped and horizontal to the skin, but the angle in ectopic nails is more rounded, and the overall position is more vertical than horizontal. Therefore the ectopic nail grows radially to the skin surface. The ectopic nail is not structurally identical to the normal nail in other respects. The fact that spherical keratohyalin granules were present in keratinocytes under the nail plate suggests that this may be part of the original nail matrix rather than nail bed. Similarly, the area to the right of Figure 2, where the granular layer is clearly visible, may be the epidermis of the proximal nail fold.

In case 1, the fact that no clear keratin pattern could be seen in the ectopic nail plate suggests that it is different from the normal stratum corneum. The large desmosomes suggest tight adhesion of nail plate with the nail matrix. Spherical keratohyalin granules observed in keratinocytes of the ectopic nail matrix were similar to keratohyalin droplets. Spherical keratohyalin droplets surrounded by numerous ribosomes have been seen in congenital ichthyosiform erythroderma, and also in the epidermis of various other mammalian species.

Keratohyalin may be formed by ribosomes and deposited as spherical granules in areas with few tonofibrils (Suzuki, Kurosumi & Miyata, 1973). In the keratinocytes of the nail bed in our case, keratohyalin droplets were observed at an early stage of formation of keratohyalin granules.

Intracytoplasmic desmosomes were observed in nails for the first time in the present study. These structures are thought to develop by membrane incorporation and dissolution in cells with active membrane turnover (Li *et al.*, 1980). Intracytoplasmic desmosomes have been seen in the epidermal cytoplasm in normal skin (Komura & Watanabe, 1975), pityriasis rosea (Okamoto *et al.*, 1982), acute radiation dermatitis (Oguchi, Komura & Ofuji, 1978), pityriasis lichenoides chronica (Watanabe, Komura & Ofugi, 1977), malignant melanoma (Klug & Haustein, 1974) and other conditions.

The difference in hardness between the ectopic nail and normal nail clippings was found to be only slight. Several variable factors such as water content influence nail hardness (Forslind *et al.*, 1980) and the similar hardness of the ectopic and normal nails suggests that the ectopic nail resembles the normal nail in its biophysical properties.

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The study was presented at the 16th International Congress of Dermatology (Tokyo, 1982).

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Malignant fibrous histiocytoma developing in a burn scar

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SUMMARY

A case of malignant fibrous histiocytoma (MFH) developing in a burn scar is presented. The patient was burnt at 3 years of age and 25 years later, a rapidly growing tumour developed in the burn scar. MFH was diagnosed by light and electron microscopy and by an immunoperoxidase method. This complication of a burn scar has not been previously reported.

Malignant fibrous histiocytoma (MFH) is a soft-tissue sarcoma which frequently occurs in the extremities. According to Enzinger and Weiss (1983), the tumour has a peak incidence in the seventh decade and is uncommon before the fourth decade. Although the aetiology of MFH is unknown, several cases have been reported after radiotherapy (Hardy *et al.*, 1978; Tsuneyoshi & Enjoji, 1979; Chowdhury *et al.*, 1980; Gonzalez-Vitale, Salvin & McQueen, 1976). We present a case of a 29-year-old man in whom MFH evolved in a burn scar.

CASE REPORT

A 29-year-old man was hospitalized because of a rapidly growing tumour in his left arm in May 1983. He had sustained a second to third degree thermal burn from boiling water in the upper half of the body at 3 years of age. A pruritic atrophic scar had been present since that time. The scar had repeatedly showed eczematous change and/or haemorrhage from scratching. A painless, reddish, small tumour was first noticed in the scar in March 1982. Examination showed a pedunculated and multilobulated tumour with a haemorrhage in the scar. Its size was approximately 8·5 × 7·5 × 5 cm (Fig. 1). Because the tumour was too large to resect completely, biopsy was performed. Combination chemotherapy was given, but 3 months later metastatic tumours appeared in the proximal portion of the left arm and both lungs. An enlarged

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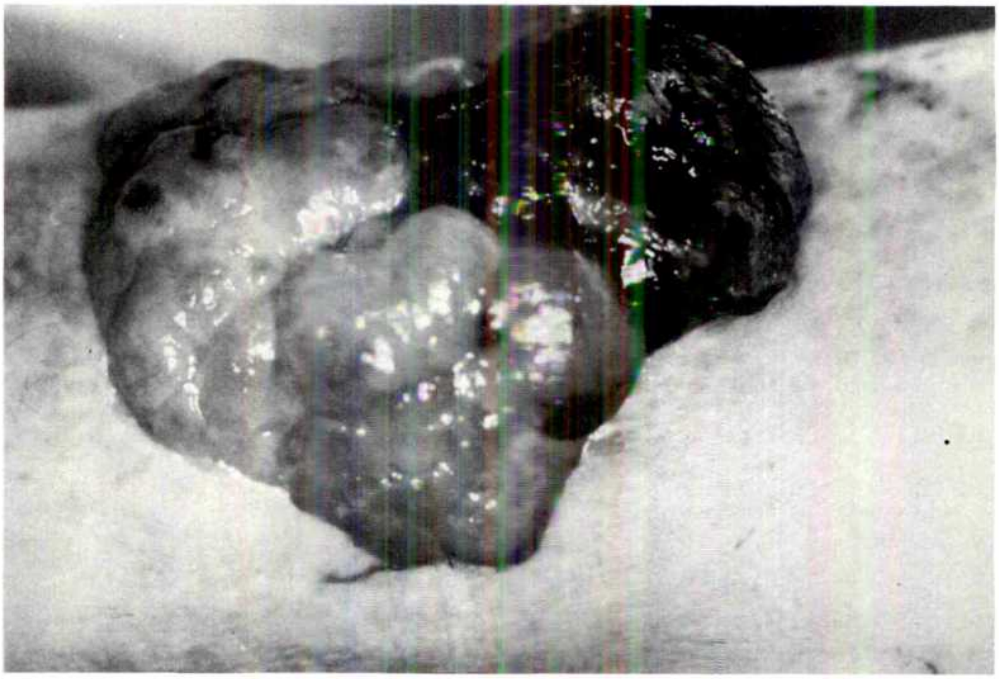

FIGURE 1. Large pedunculated, multilobulated tumour of the left arm surrounded by poikilodermatous scar.

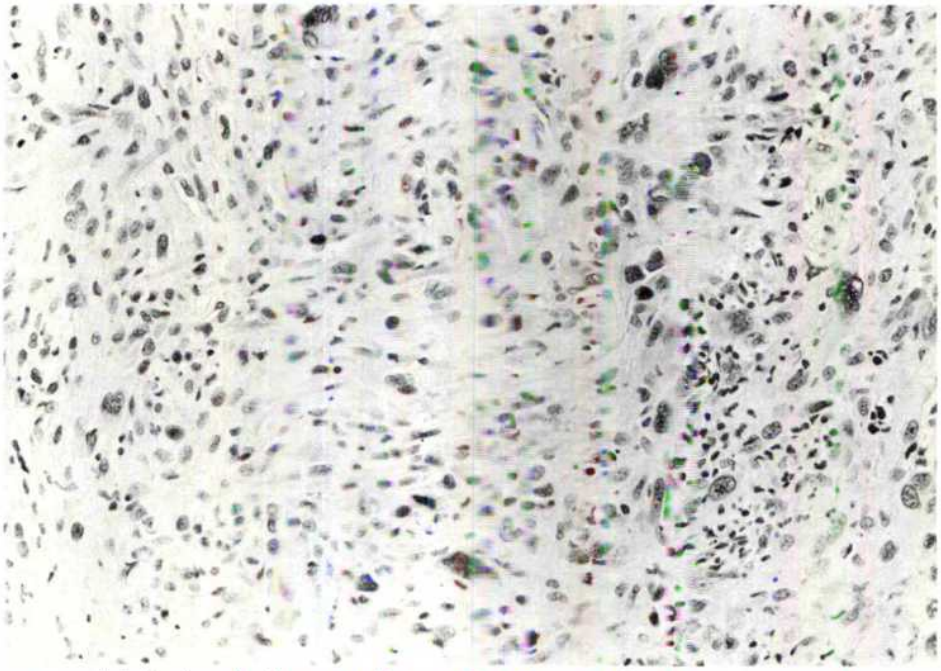

FIGURE 2. Spindle-shaped cells arranged in a storiform pattern, with mono- and multinucleated giant cells. Focal aggregates of inflammatory cells are also present. (H & E, × 204.)

lymph node in the left axillary fossa was biopsied and histological examination showed metastatic tumour.

The specimens obtained by the biopsies were fixed in 10% formalin or Bouin's solution and routinely processed for paraffin embedding. Serial sections, cut at 4·5 μm, were made for haematoxylin and eosin staining and immunoperoxidase procedures (PAP method) (Taylor, 1978). Rabbit antibodies to human lysozyme and alpha-1-antitrypsin (Dakopatts), diluted at 1:100 and 1:1000, respectively, were used for the PAP method. For control tests to absorb contaminating or cross-reacting antibodies to lysozyme and alpha-1-antitrypsin, 1 ml of anti-lysozyme and alpha-1-antitrypsin sera diluted 1:100 were tested with 0·5 mg of purified lysozyme (Green Cross Co., Japan) and alpha-1-antitrypsin (Sigma), and the supernatant was used for immunochemistry. A positive reaction was never observed.

Histologically the primary and the metastatic tumours showed a pleomorphic pattern of proliferation, composed of spindle-shaped fibroblastic cells and mono- or multinucleated histiocytic cells with pale to eosinophilic, voluminous cytoplasm (Fig. 2). Mitoses were frequent. There was a slight tendency to show a storiform pattern of proliferation. Occasionally the tumour showed a myxoid appearance with less cellularity and pleomorphism. The margin of the tumour infiltrated the surrounding stroma. By the PAP method, many tumour cells with histiocytic and fibroblastic appearances were positively stained for anti-human alpha-1-antitrypsin (Fig. 3). The intensity of staining varied, but a few tumour cells, predominantly with a histiocytic appearance, stained with lysozyme.

Ultrastructurally, the tumour was composed of fibroblastic and histiocytic cells. The fibroblastic cells were elongated with oval nuclei and the cytoplasm contained well-developed rough endoplasmic reticulum. The histiocytic cells were variable in shape with occasional

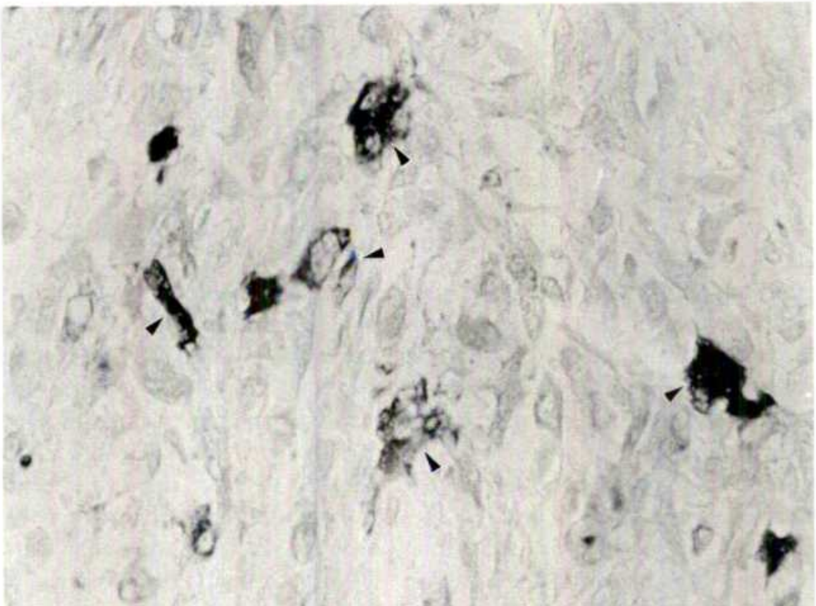

FIGURE 3. Histiocytic and fibroblastic cells (arrows) show positive staining for anti-human alpha-1-antitrypsin. (Immunoperoxidase (PAP), × 950.)

surface microvillous processes. The nuclei varied greatly in shape and contained peripheral heterochromatin clumps. The cytoplasm contained ribosomes, various numbers of lysosomes and phagolysosomes and moderate numbers of Golgi zones (Fig. 4). Intracytoplasmic lipid inclusions, autophagic vacuoles and erythrophagocytosis were seen occasionally in both types.

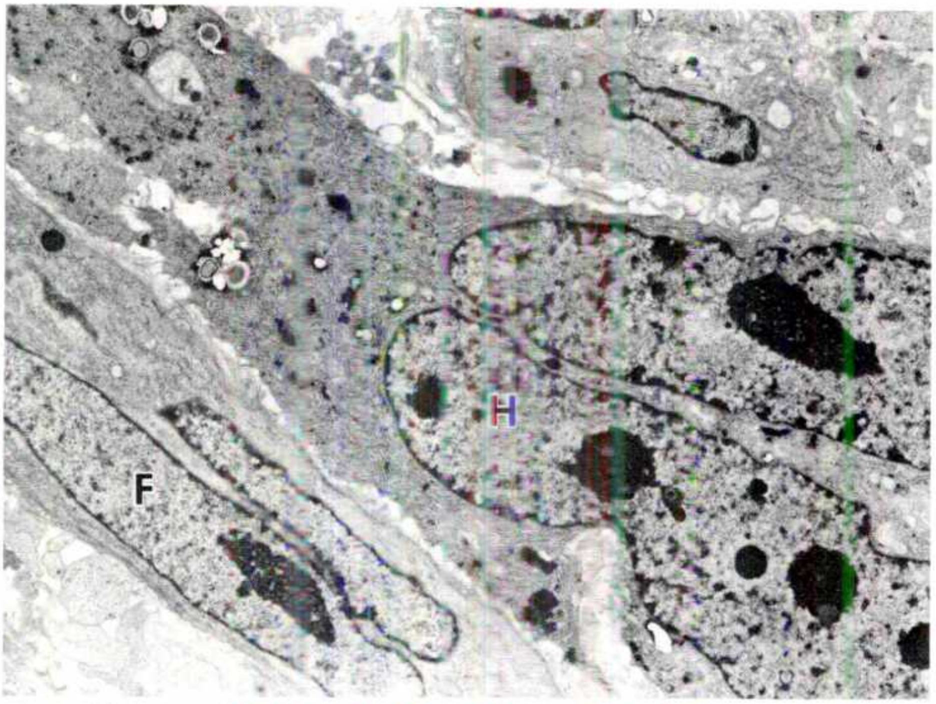

FIGURE 4. Histiocytic cells (H) with abundant ribosomes, many lysosomes and phagolysosomes, and fibroblastic cells (F) with a few lysosomes. (Electron micrograph, × 4380.)

DISCUSSION

The major problem was to decide whether the tumour represented atypical fibroxanthoma or MFH. Atypical fibroxanthoma has been referred to as pseudosarcoma of the skin, paradoxical fibrosarcoma, pseudosarcomatous dermatofibroma, and pseudosarcomatous reticulohistiocytoma. It has been claimed that atypical fibroxanthoma and MFH are indistinguishable on purely morphological grounds. However, the size and infiltrating margin found in the present tumour favoured MFH and this was supported by electron microscopy. Furthermore, the development of distant metastases justified the diagnosis as MFH. The PAP method, using anti-human lysozyme and alpha-1-antitrypsin, has become a reliable tool for the diagnosis of MFH. In the present case, tumour cells with fibroblastic and or histiocytic appearances were stained for anti-human alpha-1-antitrypsin and less frequently by lysozyme. These findings were identical with those described in the previous literature (Boulay, 1982).

MFH has been a frequent tumour among soft-tissue sarcomas (O'Brien & Stout, 1964). Approximately 50% of lesions are located in the lower extremities with the thigh being the most common site (Weiss & Enzinger, 1978). According to the histological appearance, MFH can be divided into five variants (Enzinger & Weiss, 1983). There are storiform-pleomorphic, myxoid, giant cell, inflammatory and angiomatoid patterns. The tumour in the present case showed a mainly storiform–pleomorphic pattern with a partial myxoid appearance. Ultrastructurally, the tumour was composed of fibroblastic and histiocytic cells, similar to other cases in the literature (Hardy *et al.*, 1978; Tsuneyoshi & Enjoji, 1979; Chowdhury *et al.*, 1980; Lagace, Delage & Seemayer, 1979; Alguacil-Garcia, Unni & Goellner, 1978; Miller, Kreutner & Kurtz, 1980; Gonzalez-Crussi, Wiederhold & Sotelo-Avila, 1980).

The interesting observation in the present case is that MFH developed in a thermal burn scar. Carcinoma, especially squamous cell carcinoma and less frequently basal cell carcinoma, are the most common malignant neoplasms developing in burn scars (Giblin *et al.*, 1965, Cruickshank, McConnel & Miller, 1963). Sarcomas develop rarely in burn scars and only one case of liposarcoma has been reported (Horton *et al.*, 1958). To our knowledge, no other case of MFH developing in a burn scar has been reported previously.

Four cases of MFH following radiation have been reported (Hardy *et al.*, 1978; Tsyneyoshi & Enjoji, 1979; Chowdhury *et al.*, 1980; Gonzalez-Vitale *et al.*, 1976), and the intervals between radiation and development of MFH ranged from 8 to 14 years. In these cases, MFH developed following radiation for mammary carcinoma (Hardy *et al.*, 1978; Tsuneyoshi & Enjoji, 1979), chromophobe adenoma (Gonzalez-Vitale *et al.*, 1976) and malignant lymphoma (Chowdhury *et al.*, 1980). The age of development of MFH in each case were 48, 66, 37 and 60 years. The histology was mixed storiform and pleomorphic or pure pleomorphic type. One case of MFH arising in a smallpox vaccination scar has also been reported (Slater, Parsons & Fussey, 1981). The patient received smallpox vaccination during the Second World War and MFH developed in the scar after 37 years.

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Brief Communication

Confirmation of raised phospholipase A_2 activity in the uninvolved skin of psoriasis

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SUMMARY

Phospholipase A_2 has been measured in the lesions and the 'uninvolved' skin of patients with psoriasis. We confirm a previous report that there is a generalized increase in the activity of this enzyme in psoriatic skin.

It has recently been reported that the enzyme phospholipase A_2 (PLA_2) is elevated in the 'uninvolved' epidermis of psoriatic patients (Forster *et al.*, 1983a; Forster *et al.*, 1983b). Because PLA_2 catalyses the rate-limiting step in the metabolic pathways leading to both prostaglandins and leukotrienes (Lewis & Austen, 1981), this observation could be of great importance in elucidating the pathogenesis of psoriasis (Voorhees, 1983). We have therefore repeated this investigation; in the experiments described here we have employed more stringent criteria in the selection of patients and have avoided the use of non-physiological procedures for the isolation of epidermis. In addition we have determined the activity of cutaneous PLA_2 against three different substrates.

Subjects were members of the Dutch association of psoriatic patients (Psoriasis Vereniging) who had minor lesions (mostly less than 5% of body surface) and who had received no treatment of any kind for at least 3 months. Controls were paid volunteers with no personal or family history of psoriasis. Keratotome slices (0·1 mm) were taken from lesional and uninvolved skin (at least 10 cm from any plaque). Portions of about 2 mg were weighed and homogenized in a buffer containing 10 mmol/l glycylglycine and 2 mmol/l $CaCl_2$ (pH 7·5) using an ice-cooled all-glass tissue grinder. After centrifugation (25,000 g.min at $r_{av} = 8$ cm) the protein content of the supernatant was determined by fluorescence ($\lambda_{ex} = 285$ nm, $\lambda_{em} = 340$ nm).

PLA_2 activity was measured radiometrically; the reaction mixture contained 400 nmol glycylglycine, 80 nmol $CaCl_2$, 1·6 nmol 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, approximately 20,000 d.p.m. 1-palmitoyl-2-[1-^{14}C]-oleoyl-sn-glycero-3-phosphocholine and 10 μg sodium deoxycholate in a total volume of 40 μl (final pH = 7·5). After 2 h incubation at 37°C, free fatty acid was isolated using disposable microcolumns as described previously (Feyen, van Erp & Mier, 1983) and carbon-14 determined by scintillation counting. Similar assays were carried out using the 1-palmitoyl-2-arachidonyl and the 1,2-dipalmitoyl analogues of the labelled and unlabelled phosphatidylcholine.

Our results are summarized in Table 1. It is seen that, regardless of the substrate, both lesions and uninvolved skin from the psoriatic patient show higher PLA_2 activity than the control specimens. Thus we may conclude that, despite major differences in both material and procedures, we can confirm the finding of Forster *et al.* (1983a,b) that there is a generalized

TABLE 1. PLA_2 activities (mean ± standard deviation) in keratotome slices from healthy controls and psoriatic patients. P values have been calculated using the Wilcoxon rank order test

		PLA_2 activity (pmol/h · μg protein)		
Specimen	No.	PC-2-oleate	PC-2-arachidonate	PC-2-palmitate
Control	18	1·1 ± 0·5	2·7 ± 0·8	2·4 ± 0·9
Psoriasis uninvolved	13	1·9 ± 0·7*	4·6 ± 2·2**	3·5 ± 1·0**
Psoriasis lesion	10	2·3 ± 1·1*	4·4 ± 1·3*	2·9 ± 0·9

* Differs from control at the level $P < 0·001$.
** Differs from control at the level $P < 0·002$.

abnormality of PLA_2 in the skin of the psoriatic. The only discrepancy between our data and the previous reports is that we also see a significantly increased activity in the lesion. This could be explained by our choice of intact keratotome slices rather than epidermis, since cells of the dermal infiltrate may contain substantial quantities of PLA_2.Our use of the 2-arachidonyl analogue of phosphatidyl choline (a precursor of arachidonic acid and hence of prostaglandins and leukotrienes) in parallel with the oleate and palmitate obviates any doubts as to the physiological relevance of the earlier data obtained using the 2-oleoyl analogue alone.

None the less, a major theoretical problem remains; if the clinical signs of the psoriatic lesion are to be explained in terms of increased levels of arachidonic acid metabolites, it is paradoxical to observe an increased PLA_2 activity in the 'uninvolved' skin. It seems likely that the situation *in vivo*, where PLA_2 activity is modulated by a specific endogenous inhibitor (Johnson *et al.*, 1982), is more complex than the *in vitro* findings suggest. Further work on this problem is in progress in our laboratories.

ACKNOWLEDGMENT

We thank Dr L.Heslinga, of the Organic Synthesis Department, Unilever Research Laboratories (Vlaardingen, NL) for his generous gift of the phosphatidyl choline preparations used in this investigation.

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Society Proceedings

The Anglo-Chinese Dermatological Society: Visit to the People's Republic of China—1983

It was in the summer of 1981 that Dr Celia Moss, touring China with her husband Dr Robin Ferner, slipped out of her package for long enough to make contact with Professor Wu He-Shang in Nanjing. Their talks and the visits to hospitals which followed proved to be valuable, and notable for their friendliness and goodwill. On her return to the U.K. Dr Moss gathered round herself a group of like-minded dermatologists with the aim of arranging a visit to important dermatological centres in the People's Republic of China. So was the Anglo-Chinese Dermatological Society born.

We were lucky in our officials. The genial and silver-tongued Dr John White became our first President; the meticulous Dr Robert Chalmers was our first Secretary; the astute and flexible Dr Robin Ferner treasured our funds and the menus of our banquets. Dr Peter Friedmann was our assistant treasurer, and in deference to the size of his camera became, with Dr Roger Harman, our official photographer. After many months of planning we arrived in Beijing at the end of May 1983, having visited Amsterdam for longer than we intended *en route*.

We were met by Dr Xu Wen-Yan, of the Institute of Dermatology, Chinese Academy of Medical Sciences, well known to many of us from his time at St John's Hospital for Diseases of the Skin in London, and by Mr Tao Jia-Qi of the Chinese Academy of Medical Sciences. Both stayed with us throughout our time in China and proved to be remarkable guides—friendly, knowledgeable, and patient with our endless stream of questions.

Our main dermatological hosts in Beijing were Professor Li Hong-Jiong, Head of the Department of Dermatology, the Capital Hospital, and Professor Wang Kuang-Chao, Head of the Department of Dermatology, the First Teaching Hospital, Beijing Medical College. We could have had no better introduction to Chinese dermatology. We visited both hospitals and were delighted by their judicious blend of the best of Western medicine with Chinese traditional medicine. We were impressed by Professor Wang's results using ICRF 154 for the long-term treatment of psoriasis. Our papers attracted a large audience to whom Professor Li gave illuminating summaries in Chinese. Dr John White's talk, on necrotizing fasciitis, was a reminder that this had first been described by Meleney working in the Capital Medical Hospital in the early part of this century. A ripple of applause greeted the discovery that Dr Roger Allen's slides were nearly in Chinese. Our stay in Beijing was a happy one, marred only by the sadness of Dr Edward Fox's need to fly back to England. We visited the Great Wall, the Ming Tombs, and the Forbidden City, and took in much Chinese culture and Peking duck.

From Beijing we flew the 1200 miles to Chongqing where we stayed in the splendid Hall of the People Hotel, built in the 1950s in the ornate style of the Temple of Heaven. It was there that we met many of the leading dermatologists and presented our papers at a meeting skilfully chaired by Dr L.C.Wang of the Chongqing First People's Hospital. Chongqing lies at the meeting place of the massive Yangtze and Jialing rivers, and from there we swirled down the spectacular Yangtze gorges for three days before reaching Wuhan. At the First Affiliated Hospital, Wuhan Medical College, Professor Zheng Chao-Ming and his colleague Professor Zhu showed us clinical cases and impressed us with the ingenious space-saving design of their PUVA unit, and with the success of a recent campaign to rid the area of scalp ringworm. We learned about the local prevalence of skin disease from Professor Huang Sheng-Xian of the 2nd Teaching Hospital, Hubei Medical College, and talked to a large audience at the Wuhan Medical College.

An aeroplane devoted entirely to our Society carried us onwards to Nanjing where we visited the Hospital of Chinese Traditional Medicine of Jiangsu Province; Dr Guan Feng (Chief of the Department of

Dermatology) showed us patients with general medical and skin problems being treated in the traditional way. Under supervision we practised cupping and moxibustion on each other: soon 'moxi' will be busting out all over the U.K. as many of our members later loaded themselves up with all the equipment at a local surgical instrument shop. Another high spot of our stay in Nanjing was to see the splendid new buildings to which the National Institute of Dermatology will move this year. Dr Shao Chang-Gan, Chief of the Department of Scientific Research in the Institute, told us of its many and varied research activities, and we then met staff members for an informal discussion of our different approaches to many common skin diseases. We learned a lot, and later spoke at a meeting of the Chinese Medical Association, Jiangsu branch, under the chairmanship of Professor Dai Jiyin, where we were greeted by Professor Wu He-Shang.

Having travelled by air and by water, our final run to Shanghai was by train. There we met Professor Yang Kuo-Liang, one of the elder statesmen of Chinese Dermatology, and Professor Shi Shou-Yi who showed us over the Shanghai Dermatology Institute, and outlined their numerous research projects before presenting to us a series of fascinating patients at the Hua Shan hospital. Outstanding were those with various types of amyloid demonstrated by Dr Qiu Bing-Sen. Our meeting in the afternoon was chaired by Professor Zu Zhong-Gong, 2nd Medical College of Shanghai and Professor Zhou Ding-Yao, 2nd Army Medical College.

Our 3-week visit to China ended in Shanghai. Since our return to the U.K. we have talked of little other than our tour. We await with interest the result of the present attempts to isolate and purify the active ingredients of the many traditional remedies which Chinese experience suggests are effective against skin disease. We also look forward to repaying the hospitality of our many Chinese friends when they visit the U.K.

ACKNOWLEDGMENT

Our visit was sponsored by the Chinese Academy of Medical Sciences and by the British Council. We are most grateful to them and to the numerous pharmaceutical companies, particularly Bayer, U.K. Limited, Glaxo Laboratories Limited and I.C.I. for their generous support.

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British Journal of Dermatology (1984) **110,** 735–739.

Correspondence

House dust mite avoidance and atopic dermatitis

Sir, It has recently been suggested that an important aggravating factor in atopic dermatitis (AD) may be chronic exposure to environmental house dust, with the development of a delayed hypersensitivity reaction to the house dust mite (HDM) (Mitchell *et al.*, 1982). In the light of these observations I have carried out an open trial to observe the effect of intensive efforts to reduce environmental HDM on the severity of AD.

Eighteen patients (aged 6 to 44 years) with severe AD completed a 6-week period of intensive efforts to reduce HDM in their home. This included regular vacuum-cleaning of the bedding, bedroom carpets and curtains, and the use of a plastic mattress cover. Patient (or parents) kept a daily diary card of their symptoms and were assessed by me every 2 weeks using a simple clinical scoring system for extent and activity of disease. All the patients had raised IgE levels and positive RAST (Pharmacia Limited) to HDM. Only four patients showed positive reactions to patch tests with HDM antigen (Bencard Limited).

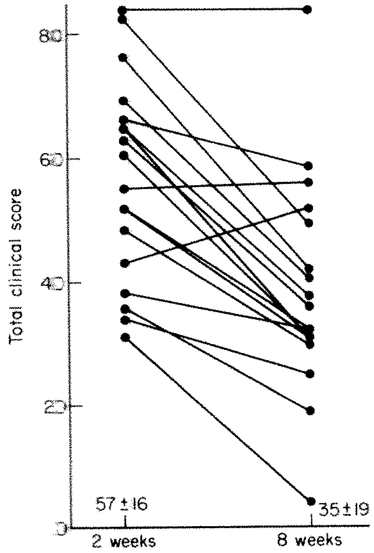

FIGURE 1. Total clinical scores recorded by the physician at the second visit and at the fifth visit in eighteen patients who completed the trial.

Fifteen patients improved, some of them dramatically and three remained unchanged or showed a slight deterioration in their eczema (Fig. 1). There was generally a good correlation between patient's and doctor's assessment.

Although this study strongly suggests that a HDM avoidance programme may be beneficial in AD, no firm conclusions can be made as it was an uncontrolled trial. As far as I am aware no controlled studies of HDM avoidance in AD have been attempted. There are various difficulties in planning such a trial and similar trials of HDM avoidance in asthma in the past has always received some criticism. These problems may be overcome in the near future as it may be possible to reduce the number of mites in the home environment, by using a fungicide spray aimed at suppressing the domestic *Aspergillus penicilloides* on which the mite feeds. I am now planning a double-blind study using such a spray to show whether or not the definite improvement observed in this trial is actually due to reduction in house dust mite or is a placebo effect.

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D.L.L.ROBERTS

U.K. qualifying examination in dermatology

Sir, The absence of my signature at the foot of the letter from Greaves *et al.* (*British Journal of Dermatology* 1984, **110**, 121–122) was the result of a definite decision and not from want of being asked. That is not to say I am happy with the present training of our junior staff, who in some ways have suffered from an increase in our teaching commitments to undergraduates, overseas students, MRCP candidates and trainee GPs. The proposed setting up of a working party is a good idea, as long as it looks at training overall and not just the introduction of an exam by those who favour one.

Some of the points dealt with by Greaves *et al.* are worthy of comment.

(1) The introduction of a curriculum to encourage a structured training programme is a separate issue from that of an exam, and, were it desirable, either could be established without the other. The practicalities of covering this by, as they suggest, pooling nationwide the facilities of the centres of excellence seem unsurmountable. Jobs which involve rotation between different parts of the country are unpopular, and presumably are not envisaged; an alternative would be compulsory attendance at a number of 'tailor-made' courses.

The financial implications of this would be considerable and the burden is unlikely to be borne by local or national bodies. Moreover there would be even more occasions than there are already when departments would be depleted of junior staff away on courses.

(2) It is important that trainees do reach a certain standard before they are appointed consultants, but this is assured to some extent by the existing recommendations; if the problem is merely that these are not comprehensive enough or sufficiently strictly enforced, there are solutions other than introducing an exam. Some flexibility in training is desirable for, in spite of its relatively small size, our speciality is one of diverse interests and jobs to which individuals bring their own skills, or the lack of them: the thought of an exam turning out dermatological 'look-alikes' is not an attractive one. What is perhaps more worrying is the prospect that, whatever is said now, the existence of an exam will be used as a lever by those who feel that it is not necessary for dermatologists to have the MRCP, and that what we really need instead is a college of our own. This could only harm dermatology and recruitment.

(3) The argument that other specialities may jump on our band waggon shouldn't deter us from doing what we think is right. It's difficult to visualize how prestigious the new diploma would be, but a number of diplomas like the DCH have been in existence for a long time without much evidence of band-waggon jumping.

(4) Greaves *et al.* seem to imply that, as not many trainees do research now, it does not matter if fewer still do it in the future: I hope this was not intended. If doctors have to wait to be motivated towards research by their training course it will be too late for most of them, unless we are to have a new breed of late developers starting research as consultants, and this seems unlikely.

(5) If there is to be an exam, all doctors entering specialist training must pass it: having it in stages seems unlikely to ease the difficulties of those who fail, although it may waste less of their time, if they are found to be not of consultant dermatologist material, to know this part of the way through rather than at the end. What will happen to the failures and how will other specialities they then try to enter consider their Dip. Derm. (failed)?

It is easier to see faults in other people's attempts to improve things than to improve them oneself, but we already have in existence a system which if found wanting, could be modified without losing its present structure. The onus is on those who want an exam to convince the rest that the result will be better dermatologists and a better speciality for them to work in. Unfortunately, we cannot do a controlled trial, and if we do introduce an exam, we shall never know if it makes things better than they otherwise would have been.

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JANET MARKS

U.K. qualifying examination in dermatology

Sir, I read the recent letter (*British Journal of Dermatology* 1984 **110**, 121–122) signed by seventeen of the luminaries of the U.K. dermatological world with interest and a good deal of sympathy. Some were signatories of a letter sent to the President of the B.A.D. some years ago stating the disquiet felt by many dermatologists about the training facilities in this country. This led to the creation of a working party to investigate this problem and the report from this resulted in the formation of the Committee for Postgraduate Training in 1979. I was the Adviser (Chairman) of this and attended many National and Regional dermatological meetings until I retired last year. This gave me the opportunity of contacting many trainees and also gave me a broad view of the quality of training given in different parts of the country. There is no doubt that dermatology is now attracting trainees of the highest calibre and the standard of training is improving.

Before radically altering the training in this country it would be wise to consider the present system, how it evolved, how it can be improved and how the different requirements of training can be incorporated into a comprehensive programme. Before the National Health Service was established in 1948, specialists in all branches of medicine were largely trained on an apprentice system and the quality of the training depended very largely on the calibre of the consultant in charge of the department. Many of the young went abroad to work with the known leaders in their particular branch. After 1948 when consultants and junior medical staff were paid for their hospital work, it was realized that the previous training arrangements were too haphazard. Following numerous meetings, the Physicians and Surgeons established the Joint Committees for Higher Medical and Higher Surgical Training the JCHMT and JCHST. Each of these committees formulated schedules of training requirements in each of the specialized divisions of Medicine and Surgery. Each of these divisions has a specialist advisory committee (SAC) serving their own branch. The JCHMT and the JCHST, on the advice of the SACs recommend those trainees who have satisfactorily completed their training schedules to the General Medical Council for recognition and registration as accredited specialists. In spite of representations from many bodies, including the BAD, the JCHMT as yet has not made Specialist Accreditation mandatory for consultant appointment. This means that a trainee may apply for and be appointed to a consultant post even though his training has neither been approved nor even considered by the SAC.

The other great weakness in dermatology in the present system is the inspection of training posts. This is arranged by the JCHMT to decide whether or not a particular post is approved for specialist training. At present only Senior Registrar (SR) posts have been inspected and unfortunately there is no doubt that in some instances the inspecting team has given approval to posts which do not conform to the basic requirements listed by the Postgraduate Training Committee. Dermatology is a small branch of medicine, most dermatologists know each other reasonably well and the inspecting teams are often on Christian name terms with the consultants in the department being inspected. Inefficient departments have been approved, albeit sometimes reluctantly. It is also unreasonable to expect the trainees to criticise adversely their training since possibly their future depends on obtaining a satisfactory reference when they eventually apply for a consultant post. A good rough guide to the training potential of a particular department is the number of applicants applying for any senior registrar vacancy. The young have their ears very close to the ground and know which training posts are good.

I agree that an examination at the end of training would probably ensure that experience in the current subbranches of dermatology is obtained. This theoretical knowledge may be acquired at the expense of gaining practical exposure to the management of individual patients. Presumably all the signatories of the letter now have this expertise without being tested by examination.

It is suggested that the trainees attend for instruction in superspecialized centres. This is undoubtedly a good idea and is listed in the postgraduate training handbook. One great difficulty arising from such attachments, together with the anxiety of working for a crucial examination in the third decade of life, is the consequent disruption of family life, since many of the trainees are by then 'young marrieds'. The B.A.D. might have to sponsor a marriage guidance service. The letter skilfully glosses over the fate of those who fail the proposed examination.

Before trying to introduce a disruptive preconsultant examination, every effort must be made to improve the existing training system which, with all its faults, has produced some of the leading dermatologists in the world. It is imperative that we should be much more realistic and even ruthless in giving approval to training posts. The SAC must continue to exercise full control over the experience acquired before specialist accreditation is granted, and of the utmost importance, nobody should be short-listed for a consultant appointment unless he or she is an accredited specialist.

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R.VICKERS

Examining Dermatologists

Sir, Dowd and Lovell's questionnaire (*British Journal of Dermatology* 1983, **109,** 235) showed that only 16% of consultants and 8% of registrars were in favour of a professional examination in dermatology. It is not surprising that such an unequivocal shout for sanity elicited a squeal for succour from the misunderstood minority for examination husbandry (Greaves *et al.*, 198[illegible]). What is the problem these closet torturers pretend will be solved by a procedure which, at the time they themselves were last subjected to it, they considered to be the absurdity that the rest of us still remember it to be? Savoyard-style let us enquire whether the punishment fits the crime.

They tell us the problem is the 'considerable unevenness in dermatological training between different centres, and exposure to some of the sub-specialties of dermatology is minimal in some places' and that 'existing training systems are poorly equipped with the background knowledge to assimilate these advances [in the biological sciences] into dermatological practice'. Assuming, which I don't, that these are the real problems (unevenness a problem, isn't it good that there will always be tall Greaves for short Shusters to look up to?), then how in the name of the unnameable will the abracadabra of an examination improve them? How will it make a mere dermatological Robin into a Bateman? The inadequacy of their answer is of course obvious even to our trainee inquisitors because they deviously try to solve the problem another way: 'It should be incumbent upon the Board responsible for the examination to organize a training programme'. Now at last we've got to it. The framed, italic-scripted diploma isn't really the answer; in fact the real purpose of the Examination is to create an Examiners' Board, and the purpose of the Examiner's Board is to create a Training Programme. But if, as analysis of their argument shows, training is really the objective, why not cut the seventeen flabby middle men and go straight for it?

Here of course our minority spoke-persons get stuck in the wheel of practicality. Who will pay for a national training scheme? Who will pay for the travel and the dermatological deputizing service essential to it? Surely they don't expect the new slenderized NHS to pay? Or do they intend to privatize the venture and take in bids from rival pharmaceutical firms? And who will decide which centres fall into the pool of excellence, other than the ones from which all the signatories come? To divert from the major impracticalities they agonize over a few trivial ones like: 'the fate of the candidate who fails the examination'. Their solution is instructive: rather than suggesting that he takes up a simpler option like internal medicine, they feel it would '. . . be wise to take the examination in stages'. Instead of solutions we already have 'bureaucrasia', the autonomous multiplication of bureaucratic neoplasia. Plans for academic hoods and gowns trimmed with vermin may already be in train.

No amount of examining will solve the problem that our trainees (a) are working apprentices, (b) are scattered throughout the land (nor am I sure it really is a problem). But if *et al.* and Greaves really want to spend their time and energy overcoming the consequences of our history and geography, they would do better organizing courses and intellectual nosh-ups than bumbling, blustering and baublising with the trivia of paper tests. Perhaps they will; after all, their endearing characteristic is they were so well trained by the old system that they want to make things even better.

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SAM SHUSTER

REFERENCE

GREAVES, M.W., IVE, F.A. and 15 others (1984) U.K. qualifying examination in Dermatology. *British Journal of Dermatology*, **110**, 121.

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Book Reviews

Dermatological Formulations: Percutaneous Absorption. By B.W.BARRY (1983) Vol. 18, *Drugs and the Pharmaceutical Sciences*, series. New York: Marcel Dekker. Pp. 480. Price S Fr 149.

The art of empirical formulation of dermatological preparations—and I use the term in no derogatory sense—was highly developed in the great European centres of dermatology in the last century. Many of the formulae evolved then, perhaps by trial and error, continue to be used successfully and even to receive qualified acceptance—if not overwhelming acclaim—by pharmacologists and pharmacotoxicologists. This period culminated in Polano's remarkable book on *Skin Therapeutics* in 1952 (and which I am delighted to see is about to re-appear). But topical pharmacology, stimulated by an increasing awareness of the importance of percutaneous absorption, especially of corticosteroids, became a complex and highly specialized science, with physics as its household god. Though we are fortunate in having a number of investigative dermatologists who have been in the forefront of developments in topical dermatopharmacology, much of the impetus of original work has passed to a great extent to University departments or drug and cosmetic houses, who often have excellent facilities and first-class workers in the field.

The author of this very detailed and comprehensive book, the latest in an honourable line of such monographs, is Professor of Pharmacological Technology at the University of Bradford. He has brought together the existing information on the theory and practice of dermatological formulation. But the key to the contents lies in the second part of the title. After a very well written and accurate introductory chapter which, so to speak, sets the scene, he discusses skin transport and the factors that influence percutaneous absorption. These, and the fifty pages devoted to the formulation of dermatological vehicles could be read with advantage by all clinical dermatologists. Other chapters are more specialized and rely on a knowledge of physics which I am not competent to interpret but there is no doubt that the author has explored in great depth problems of rheology and the physicochemical properties involved in percutaneous absorption. Professor Barry indicates that the book is aimed at pharmacists, cosmetic scientists and 'investigative dermatologists'. But I think it should find a place in the library of the Skin Department or Pharmacy of any centre interested in the art of accurate and exact prescribing. If it brings the dermatologist and his local pharmacist more closely together than is usually the case, it will have achieved an added bonus.

D.S.WILKINSON

Topics in Adolescent Medicine. Edited by R.B.Shearin (1985) Chicago: Year Book Medical Publications. Pp 308. Price £26.40.

Adolescent medicine can claim to be a 'Cinderella' subspecialty of both paediatrics and adult medicine. This book is a special edition of the Year Book series and is constructed in their familiar format. The fifteen sections cover major topics of adolescent health care. Each section begins with an overview provided by the editor, followed by year book abstracts selected from current editions of the twenty-four medical specialty and dentistry year books. Cutaneous medicine is covered by the sections on infectious diseases and dermatology.

It is difficult to see a place for this book in the dermatologist's library, as the dermatology Year Book series contains all these articles plus many more. However, for those who attract a large number of adolescents into acne clinics there are one or two useful articles in the general topics section on 'lowering broken appointment rates' and 'Why teenagers come for treatment' which may help us to improve follow-up rates in long-term studies.

N.B.SIMPSON

Aspects of the Tongue. By Robert Froriep (1828) Edited and translated by C.Garton and J.D.Gerencser, with the assistance of A.J.Drinnan (1982) Buffalo: University of Buffalo. Pp. xix, 218.

Diseases of the tongue, like other orificial diseases, fall within an area of overlap of several specialities and tend therefore to be neglected by all of them. Froriep's book on the tongue has long been regarded as a classic.

Robert von Froriep (1804–1861), born at Weimar, graduated in medicine at the University of Bonn, held appointments in surgical anatomy at Berlin and then returned to Weimar. His book on the tongue has not previously been translated into English. In the present volume the Latin version and the translation are conveniently presented on facing pages, and the original references have been identified and carefully annotated. The book will be of great interest to medical historians.

Arthur Rook

Normal and Abnormal Epidermal Differentiation. Edited by I.A.Bernstein and the late M.Seiji (1983) Basel: Karger. Pp. 344.

This record of the third Japanese-American seminar on the epidermis, held in Tokyo in 1982, constitutes Volume 11 in the series *Current Problems in Dermatology* edited by J.W.H.Mali. Contributions are mainly from Japan and America, plus one each from Cardiff and Adelaide. There is some overlap with Volume 10, which recorded the second seminar in 1979, and several authors write again on the same topic. Yet I detected little real redundancy in their articles and generally this is a useful and well-produced collection of up-to-date pieces. The 344 pages and 200 figures make it better value for money than some earlier volumes in the series.

The topics include keratins (rather less than in Volume 10), and other cytoskeletal proteins (Fuchs), calcium (Hennings, Yuspa and Holbrook) and adhesion (Karasek), while the abnormalities include Darier's and Hailey–Hailey's diseases (Ishibashi), epidermolysis bullosa (Goldsmith and Briggamon), experimental carcinogenesis (Yupsa) but not, surprisingly, psoriasis. There is a great debt to keratinocyte culture: Suzuki and his colleagues provide interesting high voltage electron micrographs of whole cultured cells, while Taniguchi and Hirone show that epidermal cells cultured on type I collagen for several weeks produce a continous basal lamina with immunoreactive type IV collagen but no anchoring fibrils. Kitano *et al.* found a wide range of inter-division times in basal keratinocytes *in vitro*, the average of 26 hours covering ranges of 13 to 69 hours and some cells never divided at all. Keratohyalin's many facets are represented by papers describing this material in hamster cheek pouch, histidine-rich (*and* cystine-rich) protein, and zinc-binding protein. If keratohyalin is a scarred battleground, trichohyalin would be virgin territory but for the efforts of George Rogers, who contributes an assay for the peptidyl arginine deaminase which converts arginine to citrulline within the trichohyalin granules of inner root sheath and medulla. Hair growth crops up less tangentially in the paper by Ogawa and Hattori, but some readers familiar with rodent hair growth may remain sceptical that shaving can induce anagen in resting follicles. Altogether this is a stimulating volume which students of the epidermis will want to read.

G.C.Priestley

British Journal of Dermatology (1984) **110,** 743–744.

News and Notices

Medicine and Mycology, Cambridge, U.K., September 1984

A Course on common and important fungal infections will be held at Fitzwilliam College, Cambridge on 26–28 September 1984. The Course will be of value to clinicians in various disciplines, and it is not primarily concerned with the technical aspects of identification of fungi. The Course emphasizes the definition of the clinical problem, and the role of the laboratory in diagnosis and management. The fee including accommodation and meals will be £127.50. Further details may be obtained from the Postgraduate Centre (Med. & Myc.), Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. (Tel. 0223-212216).

Dermatopathology Self-Assessment Workshop, Cleveland U.S.A., October 1984

A dermatopathology self-assessment workshop will be held at the Cleveland Clinic Foundation, Cleveland, Ohio, on 13 October 1984. For further information contact Dr Wilma F. Bergfeld, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44106, U.S.A.

Practical Dermatology Course for Registrars and Senior Registrars, Oxford, October 1984

This Course which is open only to trainees, will provide instruction on investigation and management in the fields of vascular disease, psoriasis and related diseases, cryosurgery and disorders of hair and nails. Each section will include lectures and clinical demonstrations. The Course will be held in the Department of Dermatology, The Slade Hospital, Oxford on 25 and 26 October 1984, and the fee will be £40. For further details apply to Drs Ryan and Dawber, 'October Course', The Slade Hospital, Headington, Oxford (Tel. 0865-64841, Ext. 499).

European Society of Paediatric Dermatologists, West Germany, October 1984

The First Congress of the European Society of Paediatric Dermatologists will be held in Münster, West Germany, on 4–7 October 1984. The main topics are immunodermatoses of childhood, diagnosis and treatment of eczemas, genodermatoses and diseases of the hair and nails. For further information please write to: Prof. Rudolf Happle, Department of Dermatology, Von-Esmarch-Str. 56, D-4400 Münster, F.R.G.

Bill Reed International Travelling Fellowships

Three fellowships will awarded this year as follows:

(1) The winner of the Poster Session at the annual meeting of the European Society for Dermatological Research will be awarded $800.00 to help support a trip to attend the annual meeting of the American Academy of Dermatology.

(2) The winner of the Stelwagon Prize for the best paper presented at the Resident's Forum at the annual American Academy of Dermatology meeting will receive an $800.00 award to support a trip to Europe where the awardee will present a paper at the annual meeting of the British Association of Dermatologists in England. Further travel to European centres can also be arranged.

(3) A third award of $800.00 will be given to an academically orientated dermatologist in the private

practice of clinical dermatology, who has been out of training no more than 5 years. It is meant to support travel for educational purposes and to broaden contacts with dermatological investigators. Anyone interested in applying for this award should send their curriculum vitae and a covering letter indicating how they plan to spend the award funds, to the Chairman of the Committee on International Affairs of the A.A.D. at the Academy office, 1567 Maple Avenue, Evanston, Illinois 60201, U.S.A. The deadline for submission is 1 September 1984. Supporting letters from abroad relevant to the planned travel are desirable. The award will be announced in December and will be based on the originality and productivity of the applicant as well as the merit of the travel plans for continued clinical investigation.

In addition, funds are available to aid young investigators to travel to another laboratory to undertake a specific, collaborative project of 1–3 months. For particulars contact William L. Epstein, M.D., Dept. of Dermatology, University of California, San Francisco, CA 94143, U.S.A

Theodor Nasemann Scholarship for Dermatovirology

This Scholarship has been founded for the purpose of encouraging research activity in Dermatovirology. The award consists of a grant of DM 2000 and a 3-month Research Scholarship. It will be awarded every 2 years to a European aged less than 40, who has published a commendable paper in Dermatovirology. Nominations are to be submitted by 1 July 1984, to Dr Theodor Nasemann, Direktor der Universitats-Hautklinik, Martinistrasse 52, 2000 Hamburg 20, W. Germany. For further information please contact: Medical Press Service, Boehringer Ingelheim Zentrale GmbH, D-6507 Ingelheim, W. Germany.

Photodermatology

A new journal, *Photodermatology*, was launched by Munksgaard International Publishers Ltd, in February 1984. The Journal will deal with all dermatological problems related to light or UVR.

Aspen Summer Skin Seminar, Aspen, U.S.A., July 1984

The Aspen Summer Skin Seminar will be held at Aspen, Colorado, on 10–14 July 1984. The registration fee is $285. For further information please contact: Joann Bauer, The Office of Postgraduate Medical Education, The University of Colorado School of Medicine, 4200 East Ninth Avenue, Box C-295, Denver, CO 80262, U.S.A.

Correction

Book Review. *Martindale: The Extra Pharmacopoeia* 28th edition. The price of this book, reviewed in the December issue Vol. 109 p. 725, should have read £60 ($130) and not £130.

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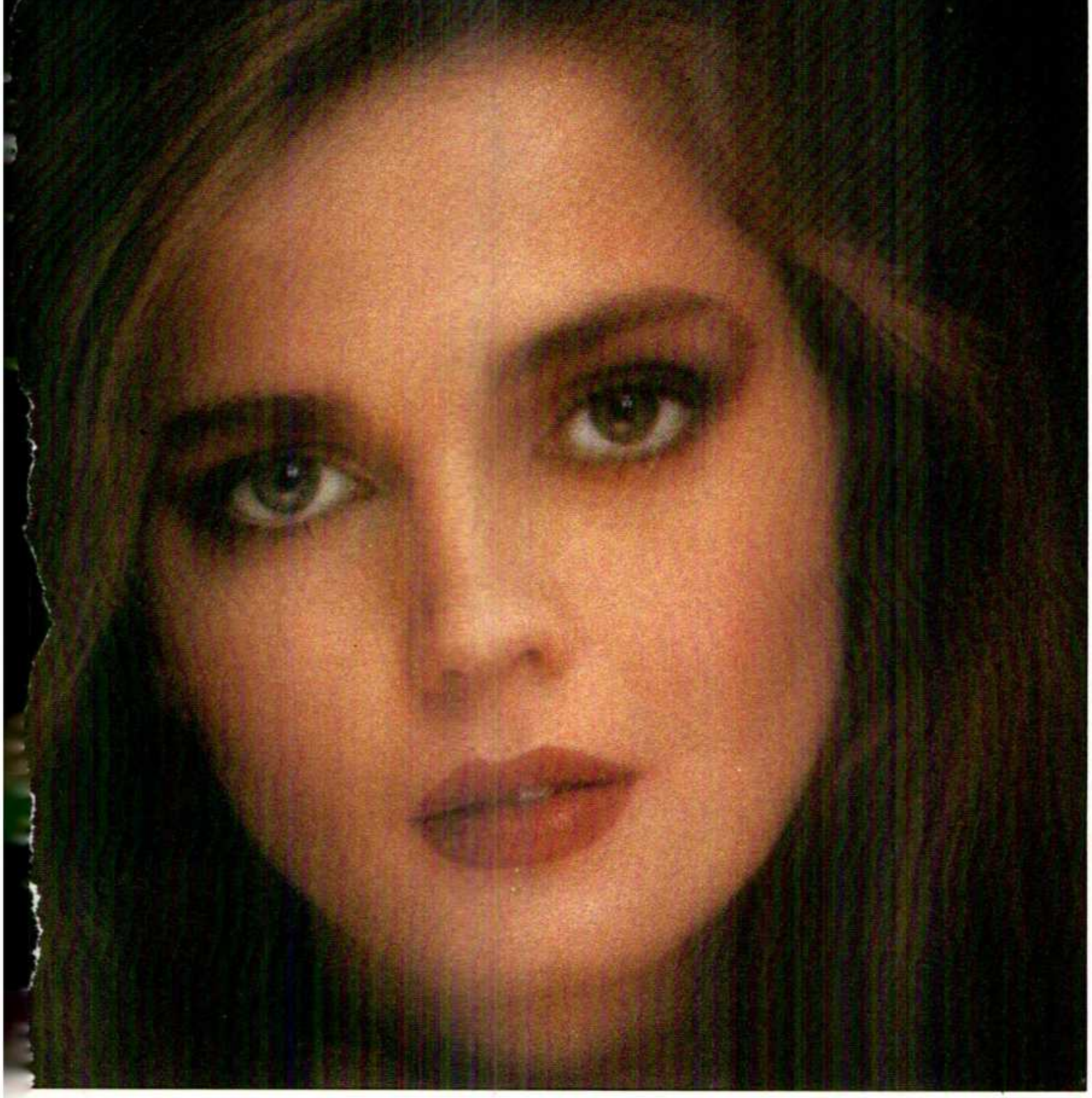

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In chronic psoriasis, Alphosyl offers the effecti-
ıess of coal tar with its direct antiproliferative action
epidermal cells, together with the benefits of allantoin
ich helps remove plaque scales.

Alphosyl, available as a Cream, Lotion and
mpoo (Alphosyl Application PC) is therefore the
l maintenance therapy for most types of chronic
iasis.

ACCEPTABLE TO PATIENTS

Unlike crude coal tar preparations, Alphosyl is easy to apply, has an unobtrusive smell, and is formulated to minimise staining.

Additionally, unlike dithranol, Alphosyl will not damage healthy skin. This combination of effectiveness and cosmetic acceptability encourages patient compliance and simplifies the management of chronic psoriatic conditions.

ALPHOSYL CREAM AND LOTION

(coal tar 5%, allantoin 2%)

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Presentation LOTION AND CREAM. Containing refined alcoholic extract of coal tar 5%, allantoin 2% in a greaseless vanishing-cream base. The lotion is a light free-flowing emulsion and the cream is smooth and homogeneous, light tan in colour. **Uses** Dermatological stimulant for the treatment of psoriasis. **Dosage and administration** Apply liberally two to four times daily, massage well into the affected areas. Occlusive dressings are unnecessary. **Contra-indications, warnings etc.** For external use only. Discontinue if irritation occurs or in cases of sensitivity to coal tar. **Pharmaceutical Precautions** Do not refrigerate. Store at room temperature. **Legal category P. Package quantities/Basic NHS Cost** ALPHOSYL LOTION. Bottle of 250ml., £2.31. ALPHOSYL CREAM. Tube of 75g., £1.59. **Further information** Alphosyl Lotion is the basic treatment for psoriasis of the body and scalp. Alphosyl Cream is designed especially for lubrication in the intertriginous areas. **Product Licence Nos.** Alphosyl Lotion 0036/5008. Alphosyl Cream 0036/5006. Further information is available on request. Stafford-Miller Ltd., Professional Relations Division, Hatfield, Herts. AL10 0NZ.

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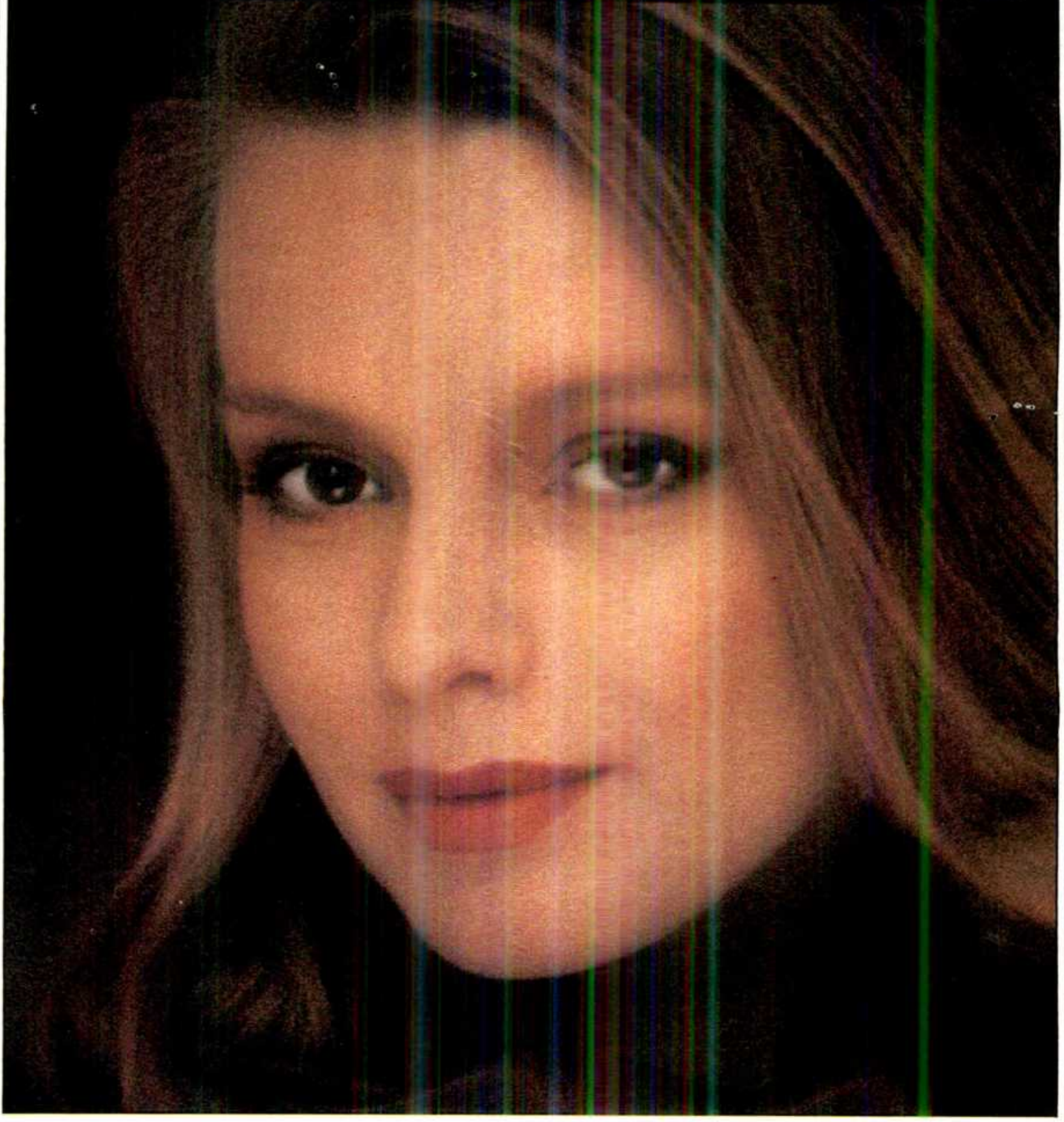

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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